# Acta Medica Scandinavica



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# IN MEMORIAM

## ERIK JOHAN WARBURG

*February 3 1892 – October 30 1969*



On October 30 1969 Erik Warburg died after a palliative operation following a period of illness

In 1916 he graduated in Medicine at the University of Copenhagen and soon joined the Hasselbalch Laboratories at the Finsen Phototherapeutic Institute where his doctoral thesis *Carbonic acid compounds and hydrogen ion activities in blood and salt solutions* was produced (1922) It was a work which was advanced for its time and may now be denoted as a classic

Warburg thought for a time of continuing with biochemical and biophysical studies but chose to take a course of internal medicine The nucleus of this training was the six years he spent at Department II of the Copenhagen Municipal Hospital under H J Bing

In 1931 he won by competition the Chair of Theoretical Medicine and became Head of the Medical Outpatient Department of Rigshospitalet and in the next year he followed Knud Faber as Professor of Clinical Medicine and Chief Physician of Medical Department B of Rigshospitalet He held these posts until retiring age in 1962

During the whole of his time as Professor Warburg was a very active participator in the life of the Faculty He was a member of the Supreme Governing Body from 1943 until his retirement He was pro-Vice Chancellor during the years 1954–55 and Vice-Chancellor 1956–58

The Danish Cardiological Society and the Danish National Heart Foundation were founded at his initiative in 1960 and 1961 and he was the first chairman of these associations

He was Horonary Member of the Danish Society for Internal Medicine the Goteborg Medical Society and the Danish National Heart Foundation He was Honorary Doctor of Oslo University

Warburg was on the board of a large number of foundations i a the Rask Ørsted Foundation and the Danish State Research Foundation of which he was chairman in 1957–58

He did very significant work on the Pharmaceutical Commission on the Specialty Committee of the Pharmaceutical Commission and later on the Specialty Board of the National Health Service from which he retired in 1964 All of these posts most of which were far from being sinecures he held at the same time as he carried on his scientific work and continued to be an inspiring leader in his department as evidenced inter alia by the large number of doctoral theses produced in the department in the course of the years In addition he ran a specialist practice which continued until he had to give it up owing to illness a few months before his death

Warburgs clinical interest soon became centred on cardiology even though the never called himself a cardiologist. In recent years it was hardly because he doubted the necessity of specialization of internal medicine He was the first in Scandinavia to describe the clinical and electrocardiographic picture of the acute myocardial infarction and thus directed attention to a clinical entity which is now only too often encountered It is however characteristic of him that at the same time as he drew attention to the disease he penetrated into its history and brought to light both Malmstens earlier cases and Obrastzows and Straschesko s work which came before Herricks (it was Herrick who was regarded throughout the world as the "discoverer" of the disease) This work was a result inter alia of his interest in and understanding of the significance of elec

trocardiography His interest led to practical and theoretical work on the physical conditions for recording of distortionless curves—a sign of the independent penetration of a subject which always stamped his scientific work and which he im printed on his students

In 1938 Warburg described in the monography *Subacute and chronic pericardial and myocardial lesions due to non penetrating traumatic injuries* (Levin & Munksgaard Copenhagen and Hum phrey Milford London) the significance of the non penetrating trauma for cardiac ailments It was not particularly well received to start with Now when through the many traffic accidents among other factors the traumatic heart diseases have come into the limelight it is cited more and more often

His great interest for and insight into chemical and physical sciences stamped his own scientific work and the fields of work which were taken up in his department He assisted Hevesy in the first experiments in the use of radioisotopes in medical science and he and his department were also well equipped to take part in the development within cardiology which followed immediately after the second world war

Warburg s interest in medicine however was ır from being limited to cardiology He always ɬ a great interest in endocrinology and ııs last work which was also the subject of his farewell lecture dealt with Klinefelter s syn drome to the description of which he added an original contribution concerning the fertility of these patients

He regarded the history of medicine with great veneration as appears from several of his pub lications This interest was in part a manifesta tion of the penetration of a subject which was always characteristic of him It is therefore not so remarkable that the often had to relegate an ap- parently well merited eponym to a more modest place in the line of discoverers" This applies to both Botallus and Fallot The latter thanks to Warburg must now often share the honour with Nicolaus Steno

His wide ranging medical interests would have been a sufficient fı ld for most to cover But not for Warburg He was insatiable in his craving to understand everything connected with human ac tivity and in fields such as politics economics and other social sciences he could propound well founded opinions to which the experts lent a ready ear In his later years his writings inclı reflections on life against the background of own scientific philosophy but with the phys understanding of the many irrational ᵽ which characterize human life He was an participator in discussions in the scientific cieties an always spirited and entertaining nent at doctoral disputations a captivating turer and an elegant after-dinner speaker

Through these various activities of which have been able to give some inkling he was ▸ by all Scandinavian physicians and—for a pl cian—a very wide section of the Danish tion But for those who got to know him u more intimate circumstances this picture was complete Despite his constant activities he time for his individual colleagues both things went well for them and—esp ciall, they went ill

That Warburg had the courage of his tions was evident from his public His courage in taking up the cudgels for others a difficult situation whether a colleague or patient is best known by the individual but ı worth emphasizing that he did so without for the unpleasantness involved for himself he would not give up as long as he b lieved a cause His personal physical courage was › during the German occupation of Denmark w by luck and resourcefulness he escaped mu at the hands of the occupying power and never less remained in the country went about his and looked after his patients during the remain of the war

One might imagine that a man who had perienced so much self-created success as E Warburg quite apart from honorary distinct and decorations would have felt convinced having used his talents in a way which both and the community could be satisfied with ˀ in his later years he fell again and again doubt as to whether he had done enough the writer of these lines or others succeeded convincing him of the contrary I must unsaid

However for those who knew him well there no doubt that he wholeheartedly and with all resources lived up to the essence of the attitı to life to which he so fully subscrib d *The n ıng of life is life*

*A Tybjaerg Hansen*

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# DIVERTICULOSIS OF THE SMALL INTESTINE AND MEGALOBLASTIC ANEMIA

*Pathogenesis*

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*Abstract* Megaloblastic anemia and small intestinal diverticulosis in a 79 year-old woman are reported Laboratory studies showed normal secretion of intrinsic factor and that the anemia was due to defective absorption of vitamin $B_{12}$ The bacterial concentration in duodenal fluid was 3 400 000 E coli per ml In vitro the bacteria had a high uptake of free $^{57}CoB$ and a low uptake of $^{57}Co$ bound to intrinsic factor This supports the theory that small intestine bacteria cause vitamin $B_{12}$ malabsorption by inhibitory action on the absorptive epithelium.

Up to 1965 megaloblastic anemia has been described in 37 patients with small intestinal diverticulosis (8) Studies of the cause of the anemia have shown malabsorption of vitamin $B_{12}$ and normal absorption of folic acid (8) Pathogenic microorganisms have been found in high concentrations in the small intestinal fluid by a few investigators (5 8) The absorption of vitamin $B_1$ is usually improved by oral tetracycline treatment (4 5) It is thus probable that the cause of vitamin $B_1$ malabsorption is closely associated with bacterial contamination of the small intestine

In 1960 Doig and Girdwood (5) showed that small intestinal bacteria in the stagnant loop syndrome had a high uptake of $^{58}Co$ labeled cyanocobalamin Bacteria isolated from diverticula in rats were shown to have a high uptake of free $^{60}Co$ labeled cyanocobalamin However the uptake was low when $^{60}CoB_1$ was bound to intrinsic factor (6) Similar studies of bacterial uptake of vitamin $B_1$ bound to intrinsic factor have not been made in patients with small intestine diverticula and megaloblastic anemia To our knowledge no study of the intrinsic factor secretion in this syndrome has been reported

## CASE REPORT

The patient, a 79 year-old woman was admitted to the hospital in April 1968 For several years she had suffered from periods of diarrhea and constipation. In the past year she had become increasingly asthenic and pale On admission she was pale and had peripheral edema The laboratory data are summarized in Table I The blood smear showed macrocytosis poikilocytosi and anicocytosis. A sternal puncture revealed megaloblastic bone marrow Schilling s test without and with intrinsic factor showed no urinary excretion of CoB in 24 hours An upper gastrointestinal X ray study (Fig 1) showed multiple diverticula of the small bowel Jejunal biopsy by the Crosby Kugler capsule showed normal small intestinal mucosa without cellular infiltration

For 12 days the pati nt was daily given 1 mg of vitamin $B_{12}$ parenterally Later on the same dose was given once a week. The reticulocyte count rose from 2.5 to 35 in 7 days hemoglobin concentration increased from 5.2 g/100 ml to 14 0 g/100 ml in 25 days

Duodenal flu d was aspirated and cultured aerobically and anaerobically The duodenal aspirate showed 3 400 000 E coli per ml the bacteria were sens tive to tetracycl ne The patient was then treated orally with daily doses of 1 g oxytetracycline for 19 days However after 14 days of th s treatment the duodenal aspirate contained more than 1 000 000 E coli per ml and the bacteria were then resistant to tetracycline After 17 days of oxytetracycline treatment the Schilling s test without intrinsic factor showed an increase to 4 9% excretion of $CoB_{12}$ in 24 h urine specimen Later checks of Schilling s test showed 0 9 % and – The fecal excretion of fats did not improve during antibiotic treatment (check 56 4 g/ 24 h)

E coli was isolated from duodenal fluid and incubated for 48 h in 100 ml Burkholder s medium (?) with 1 $\mu g$ of CoB of specific activity 0 5 $\mu c$ per $\mu g$ with and without intrinsic factor added A sterile filtrate was made by ultracentrifugation (15 000 c sec) and filtrat on of supernatant in a bacterial f lter Radioactivity was measured in a well-type sc ntillation counter in the medium befo e ultracentrifugat on and in the sterile supernatant

Table I *Laboratory data*

| | |
|---|---|
| Hemoglobin | 5 2 g/100 ml |
| Red cell count | 1 340 000 mm³ |
| Hematocrit | 17 vol % |
| MCV | 127 cμ |
| MCHC | 31 % |
| White cell count | 1800 mm³ |
| Serum $B_{12}$ | 40 pg ml (N 150–800) |
| Folic acid in serum | 7.20 ng/ml (N 3 00–11 00) |
| Folic acid in blood | 44 0 ng ml (N 35–110) |
| Serum iron | 22 μg 100 ml (N 60–160) |
| Fecal fat excretion | 42.2 g 24 h |
| D-xylose excretion in urine for 5 h | 8 % |
| Oral glucose tolerance test | Normal |
| External pancreas secretion[a] | Normal |
| Acid output[b] | Normal |
| Intrinsic factor secretion | 11 800 IF units (N 5400 25 000) |
| Schilling s test without intrinsic factor[d] | 0 % |
| Schilling s test with intrinsic factor[d] | 0 % |

[a] Analysis of duodenal contents after administration of secretin and pancreozymin (3)
[b] Augmented histamine test (7)
Intrinsic factor secretion in 1 hour after stimulation with histamine phosphate 0 04 mg per kg bodyweight (9)
[d] Urinary excretion of ⁵ Co-labeled cyanocobalamin in 24 h.
N = normal values.

Coun s were made to approximately 10 000 in each samp and the results were recorded in counts per minute ml. The bacteria had a high uptake of "free" ⁵⁷CoB₁₂, while the uptake of ⁵⁷CoB₁₂ bound to intrinsic factor (Table II) was negligible.

Table II *Uptake of ⁵⁷CoB₁₂ by E coli isolated from duodenal aspirate*

| | Radioactivity in medium before ultra centrifugation (counts ml min) | Radioactivity in sterile supernatant (counts/m |
|---|---|---|
| Medium with ⁵ CoB₁₂ and IF | 256 | 252 |
| Medium with ⁵⁷CoB₁₂ | 246 | 29 |

## DISCUSSION

The patient had a normal secretion of factor which excludes the possibility of cious anemia As folic acid concentrations in rum and blood were normal the cause of galoblastic anemia was probably malabsorption vitamin $B_1$ This was also verified by the value of Schilling s test with intrinsic factor the low serum $B_1$

Normally the small intestine fluid is sterile contains low concentrations of bacteria of pharyngeal type (10 11) and it is probable the malabsorption in our patient was due to abnormal growth of E coli (4 11) Vitamin absorption also improved during antibiotic ment That the increase in vitamin $B_{12}$ absorpt

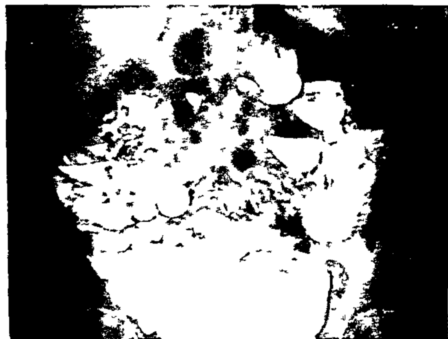


*Fig 1* Radiograph multiple diverticula throu out the small bowel.

was rath r low was possibly due to development of tetracycline resistant E. coli

Some authors favor the theory that the vitamin $B_1$ malabsorption is due to bacterial uptake of vitamin $B_{1_}$ in competition with the host (6) Our patient had a normal secretion of intrinsic factor and E coli isolated from the duodenal fluid had a negligible uptake of $CoB_1$ bound to intrinsic factor These findings do not support this theory but favor the hypothesis that the bacteria produce a factor which inhibits vitamin $B_{1*}$ absorption (1 8)

## REFERENCES


1 Booth C C & Heath, J. The effects of E. coli on the absorption of vitamin $B_{12}$. Gut 3 70 196_

2 Burkholder P R. Microbiological studies on materials which potentiate vitamin $B_{12}$ therapy in Addisonian anemia. Arch Biochem. 39 322, 195_

3 Burton, P., Evans, D G., Harper A A., Howat, H. T Oleesky S., Scott, J E. & Varley H. A test of pancreatic function in man based on the analysis of the duodenal contents after administration of secretin and pancreozymin. Gut 1 111 1960

4 Cooke W T., Cox, E. V., Fone D J., Meynrell, M. J & Gaddie R. The clinical and metabolic significance of jejunal diverticula. Gut 4 115 1963

5 Doig A & Girdwood, R. H. The absorption of folic acid and labelled cyanocobalamin in intestinal malabsorption. Quart. J Med. 29 333 1960

6 Donaldson, R. M., Jr., Corrigan, H & Natsios, G Malabsorption of $Co^{60}$ labeled cyanocobalamin in rats with intestinal diverticula. Gastroenterology 43 282, 1962.

7 Kay A W Effect of large doses of histamine on gastric secretion of HCl. An augmented histamine test. Brit. med. J 2 77 1953

8 Paulk E. A., Jr & Farrar W E., Jr Diverticulosis of the small intestine and megaloblastic anemia Amer J Med. 37 437 1964

9 Rødbro P Christiansen, P M & Schwartz, M Intrinsic factor sekretionen ved ventrikkelsygdomme Ugeskr Læg 1_8 1421 1966

10 Tabaqchali, S., Okubadejo O A., Neale G Neale G & Booth, C C Influence of abnormal bacterial flora on small intestinal function. Proc. roy Soc. Med. 59 1_44 1966

11 Tabaqchali, S & Booth, C C. Relationship of bacterial flora to absorption. Brit. med. Bull _3 785 1967

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# IMMUNOSUPPRESSIVE THERAPY IN WEGENER'S GRANULOMATOSIS

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*Abstract* The case history of a 26 year old woman with Wegener's granulomatosis is presented There were clinical signs of cutaneous pulmonary mastoidal and renal involvements. During an oliguric period of five weeks life was maintained by peritoneal dialysis Eventually she responded favourably to combined treatment with azathioprine and corticosteroids During one year's follow up the serum creatinine level has remained unchanged at about 2 mg/100 ml There has also been a marked improvement of the other manifestations of the disease and her general condition is good.

Wegener's granulomatosis is a systemic disease which mainly affects upper and lower respiratory tracts and the kidney It was first described by Klinger in 1931 (7) but was recognized as a clinical and pathological syndrome by Wegener a few years later (10 11) Up to 1967 about 200 cases had been reported The disease is characterized by necrotizing granulomatous lesions in the airways focal necrotizing glomerulonephritis and generalized angiitis

Symptoms and signs from the upper and lower respiratory tracts usually dominate in the early stages of the disease Clinical signs of renal involvement are associated with rapid development of uraemia The general picture includes fever leucocytosis and an elevated sedimentation rate

The aetiology is unknown but it is assumed that immunological mechanisms are of definite pathogenetic significance

It is the general experience that Wegener's granulomatosis is an invariably fatal disease with an average duration of 6–12 months Recent reports however indicate favourable results with immunosuppressive therapy in a few cases In these patients there have been only moderate signs of renal affection We shall therefore report a case of Wegener's granulomatosis in an advanced stage of renal failure successfully treated with an immunosuppressive regimen

## CASE REPORT

The patient is a 26 year-old married woman whose first child was born in October 1967 Her appendix had been removed in 1952 otherwise she had previously been healthy

*Early symptoms and signs*

In November 1967 the patient noticed a small tumour in the appendicectomy scar Two months later this perforated spontaneously with some secretion on the following days. In January 1968 the tumour was removed and histological sections contained necrotizing giant cell granulomas The sections did not allow any exact diagnosis Blood tests revealed a normal haemoglobin content and ESR There was however blood and protein in the urine

In the middle of February 1968 she developed symptoms of left sided otitis media Treatment with repeated paracenteses and antibiotics had no effect One month later she suddenly developed paresis of the left facial nerve and was therefore admitted to the Department of Oto Rhino Laryngology of this hospital on March 15 Upon admission signs of left-sided mastoiditis and a facial nerve palsy of peripheral type were found There was still secretion from the surgical wound on her abdomen The ESR was slightly elevated and she was moderately anaemic The urine was normal on chemical examination.

She received massive antibiotic therapy Mastoidectomy was done twice and surgical decompression of the facial nerve was attempted None of these procedures had any effect Repeated search for acid fast organisms in secretions from the ear and from the abdominal wound were negative Histological sections of the material removed during mastoidectomy contained necrotic fragments of bone and granulation tissue with signs of chronic inflammation

There was a steady deterioration of her clinical condition She was slightly febrile and the ESR rose to 114 mm X ray study of the chest revealed bilateral upper lobe infiltrations and she was admitted to one of the medical departments of the hospital

Upon admission on April 13 she complained of weakness and muscular pain She looked pale thin and asthenic Her mental state was characterized by extreme apathy Blood tests revealed normal serum creatinine and urea values The urine contained red and white blood cells Tomography of the upperlobe infiltrations was

thought to exclude tuberculosis and tumour The tuberculin reaction was now negative—she had been positive after BCG vaccination as a child

Her clinical condition was rapidly deteriorating Because of heavy nausea with vomiting and general weakness renal function tests and analysis of blood electrolytes were repeated primo May This revealed the following values potassium 6 2 mEq/l serum creatinine 13 mg/100 ml and urea 273 mg/100 ml. On May 11 she was remitted to the Renal Unit Department VII

*Treatment and course*

On admission she was anuric with serum creatinine 13 7 mg/100 ml The haemoglobin content was 8 7 g/100 ml the haematocrit 27 and the ESR 107 mm Analysis of the urine revealed protein and blood Upon microscopic examination red and white blood cells hyaline granulated and waxy casts were noted

The clinical picture suggested the diagnosis of Wegeners granulomatosis Light microscopy and electron microscopy of percutaneous renal biopsy revealed changes in accordance with this diagnosis Histological examination of biopsy from muscular tissue also suggested Wegeners granulomatosis

Peritoneal dialysis was started Simultaneously she received a combined immunosuppressive regimen with azathioprine 2 mg/kg body weight and prednisone 80 mg daily

Her condition was extremely bad through the first five weeks Dialysis had to be performed daily She received several blood transfusions and sufficient calories were provided through a gastric tube Three weeks after the start of therapy there were signs of azathioprine overdosage For a period she had extreme leucopenia and thrombocytopenia and haemoglobin values fell sharply Because of pneumonia she received massive antibiotic treatment After about two weeks the blood values allowed the immunosuppressive regimen to be resumed at reduced dosage

From the last days of June or about 7 weeks after the start of therapy her condition gradually improved The diuresis which for five weeks had varied between 500 and 700 ml rose and the renal function improved On July 1 peritoneal dialysis could be terminated X ray of the chest also revealed regression of her upper lobe infiltrations

The patient remained in the department until September 7 The serum creatinine values stabilized at 2 mg 100 ml with creatinine clearance of about 25 ml/min The protein content of 24 hour specimens of urine fell to about 2 g. The urinary sediment revealed a similar improvement, but there were still a few cellular elements and granular casts Nausea and vomiting probably related to the azathioprine therapy were noted during a short period The medication was temporarily reduced and later she has tolerated the same doses as before

At discharge from the hospital her general condition was good. She had gained 10 kg and was able to do light work. There was no longer secretion from the ear the facial nerve palsy was less pronounced and the abdominal wound had completely healed X ray of the chest still showed a small dense infiltration in the middle of the right lung There were only remnants left of the upper lobe infiltrations.

*Follow up*

The immunosuppressive regimen has been continued with azathioprine 100 mg and prednisone 15 mg daily Because of danger of azathioprine toxicity the blood values have been checked weekly Every 2–3 months she has been readmitted to the Renal Unit for more complete controls In May 1969 i e one year after her first admission to the Renal Unit, her general condition was very good Serum creatinine was 1 9 mg/100 ml and creatinine clearance 20 ml/min Still there was a moderate proteinuria, but the sediment only contained a few hyaline casts X ray of the chest revealed further regression of the infiltration in her right lung.

## COMMENTS

Evidence of the pathogenetic significance of immunological mechanisms in Wegeners granulomatosis has led to therapeutic trial of various immunosuppressive agents

The value of corticosteroids used alone is disputed (2 5) In high dosage they may provide beneficial effects in the early stages of the disease. Renal insufficiency however is not affected by this treatment The corticosteroids can at best lengthen the patient s life by a few months

During the last two years there have been several reports indicating promising results with immunosuppressive agents such as chlorambucil, nitrogen mustard and azathioprine (3 5 6 8 9) The drugs have mostly been used in combination with corticosteroids Such combined immunosuppressive regimens have in a few patients induced lasting remissions with observation periods varying from months to two years Marked improvement of the clinical state with healing of respiratory tract lesions has been noted In some of these patients there has been moderate impairment of renal function with subsequent improvement

The present patient was in an unusually advanced stage of the disease when treatment was started A combined regimen of azathioprine and prednisone was used Azathioprine is currently regarded as the immunosuppressive agent of choice in the group of antimetabolites alkylating agents and antibiotics which have been used for this purpose It is probably the most effective and certainly the least toxic of the available drugs in this group (1 4) There is ample evidence of the anti inflammatory and immunosuppressive effects

of the corticosteroids The exact mode of action of these drugs is not known Azathioprine is thought in some way to inhibit the synthesis of deoxyribonucleic acid or ribonucleic acid or both According to the literature non treated cases of Wegener's granulomatosis with renal involvement end fatally within a few months The immunosuppressive regimen used in this patient has been able to induce a lasting remission despite the presence of advanced renal failure with an oliguric period of several weeks

## REFERENCES


1 Aisenberg A. C Drugs employed for the suppression of immunologic responsiveness New Engl J Med 272 1114 1965

2. Beidelman B Wegener's granulomatosis J Amer med Ass 186 8 7 1963

3 Bouroncle B A Smith, E J & Cuppage F E Treatment of Wegener's granulomatosis with Imuran Amer J Med 42 314 1967

4 Calne R. Y Inhibition of the rejection of renal homografts in dogs by drugs Ann. roy Coll Surg. Engl 32 281 1963

5 Hollander D & Manning R T The use of alkylating agents in the treatment of Wegener's granulomatosis Ann intern Med 67 393 1967

6 Kaplan S R. Hayslett J P & Calabresi P Treatment of advanced Wegener's granulomatosis with azathioprine and duazomycin A New Engl J Med 278 239 1968

7 Klinger N Grenzformen der Periarteritis nodosa Frankfurt Z. Path. 4 455 1931

8 McIlvanie S K. Wegener's granulomatosis successful treatment with chlorambucil J Amer med Ass 197 130 1966

9 Norton W L. Suki W & Strunk S Combined corticosteroid and azathioprine therapy in two patients with Wegener's granulomatosis Arch intern Med. 121 554 1968

10 Wegener F Über generalisierte septische Gefässerkrankungen. Verh dtsch. path. Ges 29 202, 1936

11 — Über eine eigenartige rhinogene Granulomatose mit besonderer Beteiligung des Arterien System und der Nieren Beitr path Anat. 102 36 1939

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# CHLORPROPAMIDE AND LIPID METABOLISM OF RAT AND HUMAN ADIPOSE TISSUE IN VITRO[1]

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*Abst act* Lipolysis measured as glycerol release from rat adipose tissue in vitro was inhibited by chlorpropamide This antilipolytic effect was independent of the addition of glucose to the incubation medium At the same time chlorpropamide decreased the re-esterification of FFA formed by l polysis This inhibitory effect was more pronounced in tissue incubated in medium with g ucose present. Since lipolysis as well as re-esterification of FFA is accelerated by glucose present data would indicate that chlorpropamid primarily interferes with the glucose metabolism or the stimulatory action of glucos on the two pathways of the triglyceride FFA cycle Chlorpropamide (1 mg/ml) had no effect on the glycerol release and lipogenesis of human omental tissue The present findings lend no support to the recent theory that sulfonylureas decrease the plasma levels of FFA and glycerol in man by direct action of the drug on ad pose tissue

Acute administration of sulfonylurea compounds such as tolbutamide and chlorpropamide produces in non diabetic subjects and in patients with maturity-onset diabetes mellitus a prompt fall in the plasma levels of FFA (2 3 4 6) and glycerol (3) These effects have been attributed to a decrease in the lipid mobilization from adipose tissue due to insulin released by the direct action of the drugs on the $\beta$-cells of the pancreas Previous observations in this laboratory (6) were consonant with this concept since they showed that chlorpropamide in vitro had no influence upon the net release of FFA and glycerol from the epididymal fat pad of fasting normal rat

On the other hand it was reported recently by Brown and Stone (4) Stone and Brown (17) and Stone et al (18) that tolbutamide and tolazamide inhibited lipolysis in isolated fat cells and in sections of fat tissue removed from fasting normal rats It was shown in their experiments that the sulfonylurea compounds reduced significantly the net release of FFA and glycerol from adipose tissue incubated in glucose free medium This antilipolytic effect was demonstrated under basal conditions and when lipolysis was enhanced by hormones with adipokinetic action or by theophylline However the sulfonylurea compounds produced no decrease in the concentration of FFA in fat tissue which is otherwise observed under conditions of depressed lipolysis in rat adipose tissue for instance in the presence of insulin (11) or nicotinic acid (21) This suggests that the effect of sulfonylurea may not be purely antilipolytic

The present experiments were designed to elucidate the effect of sulfonylurea on lipid metabolism of rat adipose tissue and to investigate whether sulfonylurea inhibits the lipolysis also of human adipose tissue in vitro Chlorpropamide which is poorly metabolized in man (10) was used in all experiments

## MATERIAL AND METHODS

Epididymal fat tissue was obtained from rats weighing 1 0-160 g After about 20 hours of fasting the rats were killed by decapitation The fat pads were rapidly removed and placed in Krebs Henseleit b carbonate buffer (KHB) containin bovine albumin and maintained at 37 C. In some experiments isolated fat cells were prepared (14) In experiments in which the fat pads were used the fat pad of one s de was placed in medium containing chlorpropamide and the contralateral fat pad served as control

Omental tissue sections were removed from human

[1] Presented at the Annual Meeting of the Scandinavian Society for the Study of Diabetes in Stockholm on February 1-3 1968

Whereas the incorporation of labelled palmitate into neutral tissue lipids was uninfluenced by chlorpropamide the amount of radioactivity recovered as tissue FFA tended to increase with higher concentrations of chlorpropamide However this effect was significant ($p < 0 05$) only for tissues incubated in medium containing 1 mg/ml of chlorpropamide

*Sections of rat adipose tissue*

Fig 2 and Table I present the results from the experiments in which whole fat pads of fasting rats were incubated in KHB medium with (2 mg/ml) and without glucose added The glycerol release was decreased from 3 44 ± 0 03 to 2 39 ± 0 06 μmoles/g TG/2 h ($n = 6$ mean ± standard error) by chlorpropamide added at a concentration of 3 mg/ml to medium containing glucose This inhibitory effect of chlorpropamide on the glycerol release was observed whether glucose was present in medium or not and was of similar order of magnitude in both types of medium No alteration of the glycerol release was observed in medium containing 1 mg/ml of chlorpropamide Table I show the effects of 3 mg/ml of chlorpropamide on the net release of FFA the final concentration of FFA in tissue and the incorporation of radiopalmitate into neutral lipids and FFA of tissue The lower concentration of chlorpropamide (1 mg/ml) produced no changes in these parameters and these data are not charted A decrease in the concentration of FFA in tissue was observed when 3 mg/ml of chlorpropamide was added to glucose free medium The net release of FFA tended to be lower in the presence of chlorpropamide but this effect was not significant On the other hand the net release of FFA was increased in glucose-containing medium Small but consistent changes in the total recovery of

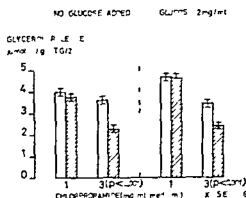



*Fig* Effect of chlorpropamide on glycerol release from epididymal fat pad of rat Statistical difference was calculated from the difference between the fat pad of one side incubated in the presence of chlorpropamide (hatched columns) and the contralateral fat pad incubated in the control medium (white columns) Fat pads were incubated in 4 ml of KHB buffer containing 4% bovine albumin, with (2 mg/ml) and without glucose added.

Table I *Effect of chlorpropamide (3 mg/ml) on the net release of FFA final tissue FFA concentration and incorporation of palmitic acid-9 10-³H into neutral lipids and FFA of rat epididymal fat pad in vitro*

Mean ± standard error of six incubations Palmitic acid −9 10− H was added in an amount of radioactivity providing approximately 100 000 counts min ml and the specific activity of FFA was about 300 000 counts min μmoles Experimental conditions are given in Fig. 2 Statistical significance was calculated from the individual difference between two whole fat pads of each rat incubated in medium with and without chlorpropamide respectively

| Added to medium | | Results | | | |
|---|---|---|---|---|---|
| Chlorpropamide (mg ml) | Glucose (mg ml) | FFA net release | Final tissue FFA con | Incorporation of palmitic acid −9 10 H: Neutral lipids[b] | Incorporation of palmitic acid −9 10 H: FFA |
| *Exp 1* | | | | | |
| 0 | | 4 31 ± 0 08 | 5 23 ± 0 12 | 7_000 ± 3100 | 14 000 ± [illegible]00 |
| 3 | 2 | 5 57 ± 0 18 | 5 14 ± 0 10 | 83 000 ± 3900 | 0 000 ± 400 |
| | | < 0 05 | | | < 0 001 |
| *Exp 2* | | | | | |
| 0 | 0 | 4 3? ± 0 18 | 8 10 ± 0 8 | 9 000 ± 1300 | 4 000 ± 500 |
| 3 | 0 | 3 34 ± 0 1 | 7 12 ± 0 27 | _1 000 ± 600 | _7 000 ± 500 |
| | | > 0 05 | < 0 05 | | |

μmoles g TG [b] Counts min g TG

Table II *Effect of chlorpropamide (3 mg/ml) on FFA metabolism of rat epididymal adipose tissue in vitro*

Statistical significance calculated from the experimental results given in Fig 2 and Table I Δ Re-esterification 3× glycerol release (exp—control)—(FFA net release (exp—control) + final tissue FFA (exp—control))

| Δ Experimental control | No glucose added | Glucose 2 mg/ml |
|---|---|---|
| Glycerol release | −1 36*** | −1 05 * |
| FFA re-esterification | −2 12 | −4 32 |
| Final tissue FFA | −0 98* | −0 09 |
| FFA net release | −0 98 | +1 26 |

μmoles/g TG
*** $p<0\ 001$ $p<0\ 01$ $p<0\ 05$

radiopalmitate in tissue were demonstrated in the presence of chlorpropamide Assuming that there was no appreciable recirculation of radiopalmitate once taken up in tissue one can calculate from the initial specific activity of medium FFA that the net uptake of FFA from the glucose free control medium was 0 14 μmoles/g TG/2 h and from medium with chlorpropamide added 0 11 μmoles If account is taken of the lesser dilution of labelled FFA with unlabelled FFA from tissue in medium with chlorpropamide added a small decrease in the uptake of FFA seemed to be produced by chlorpropamide However this effect was small and might not influence the tissue

Table III *Effect of chlorpropamide (3 mg/ml) on glycerol release and final tissue FFA concentration of rat epididymal fat pad incubated in KHB buffer containing no albumin*

Mean ± standard error of four incubations Experimental conditions and statistical calculations are the same as given in Table I and Fig 2

| Added to medium | | Results | |
|---|---|---|---|
| Chlorpropamide (mg/ml) | Glucose (mg/ml) | Glycerol release | Final tissue FFA conc |
| *Exp 1* | | | |
| 0 | 2 | 3 46±0 08 | 3 04±0 18 |
| 3 | ? | 2 49±0 05 | 3 56±0 17 |
| | | $p<0\ 005$ | |
| *Exp 2* | | | |
| 0 | 0 | 4 45±0 06 | 6 50±0 04 |
| 3 | 0 | 3 13±0 05 | 5 66±0 0? |
| | | $p<0\ 005$ | $p<0\ 0?5$ |

μmoles/g TG

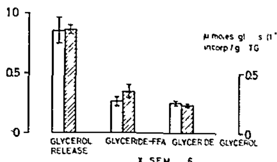



*Fig 3* Effect of chlorpropamide 1 mg/ml on the glyc release and the incorporation of 1 C glucose into ... eride glycerol and glyceride fatty acids of human omen adipose tissue in vitro Mean ± standard error of experiments Sections of omental tissue were remo from six non-diabetic subjects during surgical operati Basal incubation medium was 3 ml of KHB buffer co taining 3 % of human albumin 1 mg/ml of glucose, a 0 5 μC/ml of 1 C glucose Duplicate incubations w carried out for 2 h Statistical significance was calcula from the mean differences between tissue incubated control medium (white columns) and in medium co taining chlorpropamide (hatched columns)

concentration of FFA Likewise it can be c culated that the chlorpropamide effect on t uptake of FFA into tissue incubated in mediu with glucose present did not effect significant the tissue concentration of FFA

Table II summarizes the effects of chlo propamide (3 mg/ml) on the glycerol release t concentration of FFA in medium and tissue a the re-esterification of FFA The difference re-esterification of FFA between adipose tiss incubated in medium containing chlorpropami and in control medium was calculated as the d ference in glycerol release times three minus t difference in the net release of FFA and min the difference in the final concentration of FF in tissue Chlorpropamide significantly decrease the re-esterification of FFA in the absence as we as in the presence of glucose This effect wa more pronounced in medium containing glucos whereas the antilipolytic effect was of the sam order of magnitude in the two media A net a cumulation of FFA was observed in medium wit glucose added which will be best explained by a increased outflow of FFA from tissue in th presence of chlorpropamide since no apparen chlorpropamide effect on the uptake of FFA wa demonstrated from the isotopic data In order t

-st the possibility that the effect of chlor propamide on FFA metabolism was dependent on n effect of the drug on the transfer of FFA from issue to albumin studies were undertaken also on tissue incubated in medium containing no lbumin Table III shows that chlorpropamide 3 mg/ml) decreased the glycerol release signifi antly in both glucose-containing and glucose free nedium The effects of chlorpropamide on tissue FA concentration were also in agreement with hose observed in medium with albumin present t can be calculated that chlorpropamide de reased the re-esterification of FFA to the same extent in the two types of media

*Human omental adipose tissue*

In a number of experiments on subcutaneous and omental adipose tissue no effects on the net release of FFA and glycerol were produced by chlorpropamide when added at concentrations observed in plasma of diabetic subjects treated with this agent, i e in the range of 0 03 to 0 1 mg/ml of chlorpropamide Fig 3 summarizes the results from different experiments in which 1 mg/ml of chlorpropamide was used and when omental tissue was obtained from non-diabetic subjects during surgical operation No effect of chlorpropamide was observed on the glycerol release and on the incorporation of 1 $^{14}$C glucose into glyceride glycerol and glyceride fatty acids

DISCUSSION

The present data show that chlorpropamide at 0 1 mg/ml inhibits glycerol release from isolated rat fat cells Thus the results are consonant with the recent studies of Brown and Stone (4) in which tolbutamide was used Likewise the results con firm previous observations (6) showing that chlor propamide added at a concentration of 1 mg/ml did not decrease the glycerol release from intact adipose tissue of rat However 3 mg/ml of chlor propamide produced a significant inhibition of the glycerol release This more pronounced sus ceptibility of isolated fat cells to chlorpropamide is not surprising since it is well known that these fat cells are more sensitive to various hormones (17) than intact adipose tissue Since no data are available on the plasma concentration of sul fonylurea required to decrease the plasma levels of FFA and glycerol in rat it is not possible to decide from these studies whether or not sulfonylurea inhibits the lipid mobilization in vivo through a direct action on adipose tissue in this species However the present findings of an in creased outflow of FFA from rat adipose tissue incubated in glucose-containing medium certainly give no support for such a mechanism In man it is even more unlikely that chlorpropamide per se decreases the mobilization of FFA from adipose tissue in vivo since no effect of chlorpropamide on the glycerol release was observed in experi ments in which the agent was added at concentra tions up to 1 mg/ml This concentration is about ten times higher than that observed in plasma of diabetic subjects after intravenous administration of 0.5 g of chlorpropamide (unpublished data)

Our data show that chlorpropamide inhibits the lipolysis of rat adipose tissue only when present at concentrations which at the same time block the re-esterification of FFA Since it has been reported that in rat adipose tissue glucose stimulates not only the re-esterification of FFA but also the lipolysis (5 7) it seems tempting to attribute the apparently dual effect of chlor propamide to an inhibition of the glucose metabo-lism or its sequences related to the esterifying and lipolytic processes Conflicting data have been reported on the effect of sulfonylurea on glucose metabolism of rat adipose tissue in vitro It has been said that sulfonylureas increase (13 14) and that they have no effect on the glucose oxida tion by rat adipose tissue (8) Renold et al showed in the same experiments (13) that the lipogenesis was significantly reduced by chlorpropamide In a number of experiments we have confirmed these findings but from these uncharted data it has not been possible to conclude whether the effects of chlorpropamide on lipid metabolism are secondary to inhibition of glucose metabolism per se or whether they are localized to some step more close to the esterifying and lipolytic mech anisms Anyhow the net effects of chlorpropamide in rat adipose tissue bear a close resemblance to those of 2-deoxyglucose an agent which also in hibits the lipolysis and the re-esterification of FFA in rat adipose tissue in vitro (5)

It was shown in some of our experiments that the changes in the net release of FFA did not parallel the net changes in tissue concentration of FFA Thus the outflow of FFA from adipose tissue into glucose-containing medium was unex pectedly stimulated by chlorpropamide (3 mg/ml)

when at the same time no changes in tissue FFA were observed This discrepancy between changes in medium and tissue FFA under certain conditions has previously been reported and discussed extensively by Bally et al (1) These authors have shown that glucose did not inhibit the ACTH stimulated release of FFA whereas the tissue concentration of FFA decreased From this and related experiments it was suggested that two different pools (spaces) of FFA might exist in adipose tissue one esterifying pool and an outer pool responsible for the releasing rate of FFA from tissue to the extracellular space With chlorpropamide we have in medium with and without glucose induced various degrees of inhibition of FFA re-esterification At the same time the inhibitory effect of chlorpropamide on lipolysis was uninfluenced by the addition of glucose to medium In order to explain the findings of Bally et al as well as our own observations of an increased outflow of FFA under conditions when the inhibition of re-esterification of FFA was more pronounced than that of lipolysis we think that other mechanisms may control the rate of mobilization of FFA from tissue As an alternative hypothesis we suggest that the rate or the changes in rate of lipolysis and re-esterification of FFA is recognized by some common mechanism which is responsible for the mobilization of FFA

## ACKNOWLEDGEMENTS

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## REFERENCES

1 Bally P R. Kappeler H Froesch E R & Labhart A Effect of glucose on spontaneous limitation of lipolysis in isolated adipos tissue a potential regulatory mechanism Ann N Y Acad Sci 131 143 1965

2 Bierman E L Roberts T N & Dole V P Effect of tolbutamide (Orinase) on plasma non-esterified fatty acids Proc Soc exp Biol (N Y) 95 437 1957

3 Botterman P Schwarz K Schulze Solde R. & Dambacher M Untersuchungen uber den Fettstoffwechsel bei der Fettsucht Diabetologia 1 180 1965

4 Brown J D & Stone D B Antilipolytic effects of sulfonylurea drug on induced lipolysis in isolated fat cells of rat Endocrinology 81 71 1967

5 Carlson, L A Inhibition of the mobilization of free fatty acids from adipose tissue Ann N Y Acad Sci 131 119 1965

6 Carlson L A & Östman J The influence of chl propamide and salicylate on plasma lipids in ma In Proceedings of the First International Pharm cological Meeting Vol II Effects of drugs synthesis and mobilization of lipids p 157 B E. Horning ed Pergamon Press Oxford 1963

7 Chlouverakis C The action of glucose on lipoly Metabolism 16 469 1967

8 Ditschuneit H Pfeiffer E. F & Rossenbeck H. Über die Bestimmung von Insulin im Blute am e didymalen Fettanhang der Ratte mit Hilfe markier Glucose III Die Wirkung von Sulfonylharnstoff und Biguanid (DBI) auf den Kohlenhydratsto wechsel des isolierten Rattenfettgewebes und Ratt zwerchfells Klin Wschr 39 71 1961

9 Dole V P A relation between non-esterified fa acids in plasma and the metabolism of glucose clin Invest 35 150 1956

10 Johnson P C Hennes A R Driscoll T West K M Metabolic fate of chlorpropamide man Ann N Y Acad Sci 74 459 1959

11 Jungas R L & Ball E G Studies on metabolism of adipose tissue XII The effects insulin and epinephrine on free fatty acid a glycerol production in the presence and absence glucose Biochemistry 2 383 1963

12 Larsen J A Elimination of glycerol as a meas of the hepatic blood flow in the cat Acta physi scand 57 224 1963

13 Renold A E Zahnd G R Jeanrenaud B Boshell B R. Some effects of tolbutamide a chlorpropamide in vitro Ann N Y Acad Sci 7 490 1959

14 Rodbell M Metabolism of isolated fat cells Effe of hormones on glucose metabolism and lipoly J biol Chem 239 375 1964

15 Rodriguez Candela J L & Lopez Quijada C I fluence in vitro of tolbutamide on the gluc uptake and glycogen synthesis of the epididymal f Med exp 2 40 1960

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19 Trout D L Estes E H & Friedberg S J Tit tion of free fatty acids of plasma a study of curre methods and a new modification J lipid Res 199 1960

20 Wieland O Eine enzymathische Methode zur B stimmung von Glyzerin Biochem Z 3 9 3 1957

21 Östman, J Effect of nicotinic acid on the fatty ac metabolism of adipose tissue in alloxan diabe rats Metabolism 13 675 1964

22 Östman J A procedure for in vitro studies on fat acid metabolism by human subcutaneous adipo tissue Acta med scand 177 183 1965

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# EXERCISE PERFORMANCE AND PERCEIVED EXERTION IN PATIENTS WITH CORONARY INSUFFICIENCY ARTERIAL HYPERTENSION AND VASOREGULATORY ASTHENIA[1]

G Borg and H Linderholm

*From the Depa t ents of Clinical Phys ology and Clini al Psychology Un ersity of Umeå Umeå S eden*

*Abstract* A standardized work test has been performed by healthy subjects patients with coronary heart disease patients with arterial hypertension and with the vasoregulatory asthenia syndrome Heart frequency and rating of perceived exertion according to a rating method were asses ed at various work loads. Different measures of physical working capacity were estimated

Patients with vasoregulatory asthenia—and patients with arterial hypertension, although less markedly—rated the exertion to be less in relation to heart frequ ncy than healthy controls particularly at low rating levels On the contrary patients with coronary heart disease rated the exert on to b higher particularly at high ratings in relation to heart frequency

In all pat ent group studied there was a small r mean increas in heart rate in relation to a given increase in rating cf exertion, i e for a given increas in heart rat there was a greater increase in rating of exertion than in healthy controls

Submaximal measures of physical working capacity were based on heart rate and rating of perceived exertion The ratio between measurements of physical working capacity based on heart rate and thos based on rating of perceived exertion was low in the VA group and high in pat ents with corcnary insufficiency when compared with controls of equal age Patients with a low max mal performanc during the test also had a low submaximal physical work ing capac ty estimated from heart frequency as well as from rating of perc ived exertion

The difference found between the various patient groups, e pec ally that between patients with coronary heart dis as and patien s with the vasoregulatory asthen a syndrome is of differential dia_nost c value

In healthy subjects there is a fairly close relationship between heart rate during exercise and the subjective perception of exertion according to a rating method (3) This relationship changes with the age of the subject (5) Having used rating of perceived exertion for several years in routine tests for the determination of the physical working capacity of patients we got the impression that in some groups of patients there is a deviation from normal relationships between heart rate and rating of exertion Thus Borg and Linderholm (4) cf also Borg (3) reported that patients with arterial hypertension and coronary insufficiency rated the exertion high in relation to heart rate as compared with controls

In the present investigation different groups of patients were compared with healthy subjects of comparable age in order to further elucidate these differences At the same time different methods of estimating the physical working capacity have been compared

## MATERIAL

Groups of patients with the vasoregulatory asthenia syn drome (8) coronary heart disease and arter al hyperten on were selected from about 70^0 patients who were examined during a three year per od for d agnostic purpo es with ECG at rest, during and after exercise at the Department of Cl n cal Physiology University Hospital Umeå Con s cu ively examined patients who fulfilled the criter a to be described below were included in the study All pa t ents with dig talis med cation s gns of val ular heart disease and with ECG changes or h story typical of pre viou heart infa ction were excluded Groups of healthy age-compatib e control subjects were included in the study for omparison Some general characterist cs of the groups are g ven in Table I The pat ent groups are to a g eat e tent identical with those includ d n a study on the effects of hyperventilation on ECG (6)

[1] A report of this paper was g en at the Umeå Medical As ociat on in 1967

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## REFERENCES


1 Bally P R, Kappeler H Froesch E R & Labhart A. Effect of glucose on spontaneous limitation of lipolysis in isolated adipos tissue a potential regulatory mechanism Ann N Y Acad Sci 131 143 1965

2 Bierman, E L Roberts T N & Dole V P Effect of tolbutamide (Orinase) on plasma non-esterified fatty acids Proc Soc exp Biol (N Y) 95 437 1957

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8 Ditschuneit H Pfeiffer E F & Rossenbeck, H G Über die Bestimmung von Insulin im Blute am ... didymalen Fettanhang der Ratte mit Hilfe markierter Glucose III Die Wirkung von Sulfonylharnstoffe und Biguanid (DBI) auf den Kohlenhydratstoffwechsel des isolierten Rattenfettgewebes und Rattenzwerchfells Klin Wschr 39 71 1961

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10 Johnson P C Hennes A R Driscoll T ... West, K M Metabolic fate of chlorpropamide in man Ann N Y Acad Sci. 74 459 1959

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12 Larsen J A Elimination of glycerol as a measure of the hepatic blood flow in the cat Acta physiol scand 57 224 1963

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14 Rodbell, M Metabolism of isolated fat cells. Effects of hormones on glucose metabolism and lipolysis J biol Chem. 239 375 1964

15 Rodriguez-Candela J L & Lopez Quijada, C Influence in vitro of tolbutamide on the glucose uptake and glycogen synthesis of the epididymal fat ... Med exp 2 40 1960

16 Snedecor G W Statistical methods Iowa State College Press Iowa 1957

17 Stone D B & Brown J D Effect of sulfonylurea drugs on plasma free fatty acid and blood glucose concentration in man Diabetes 15 314 1966

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0 Wieland O Eine enzymathische Methode zur Bestimmung von Glyzerin Biochem. Z. 329 ... 1957

21 Östman, J Effect of nicotinic acid on the fatty acid metabolism of adipose tissue in alloxan diabetic rats Metabolism 13 675 1964

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Patients with vasoregulatory asthenia—and patients with arterial hypertension, although less markedly—rated the exertion to be less in relation to heart frequency than healthy controls particularly at low rating levels On the contrary patients with coronary heart disease rated the exertion to be higher particularly at high ratings, in relation to heart frequency

In all patient groups studied there was a smaller mean increase in heart rate in relation to a given increase in rating of exertion i e for a given increase in heart rate there was a greater increase in rating of exertion than in healthy controls

Submaximal measures of physical working capacity were based on heart rate and rating of perceived exertion The ratio between measurements of physical working capacity based on heart rate and those based on rating of perceived exertion was low in the VA group and high in patients with coronary insufficiency when compared with controls of equal age Patients with a low "maximal" performance during the test also had a low submaximal physical working capacity estimated from heart frequency as well as from rating of perceived exertion

The difference found between the various patient groups especially that between patients with coronary heart disease and patients with the vasoregulatory asthenia syndrome is of differential diagnostic value

In healthy subjects there is a fairly close relationship between heart rate during exercise and the subjective perception of exertion according to a rating method (3) This relationship changes with the age of the subject (5) Having used rating of perceived exertion for several years in routine tests for the determination of the physical working capacity of patients we got the impression that in some groups of patients there is a deviation from normal relationships between heart rate and rating of exertion Thus Borg and Linderholm (4) cf also Borg (3) reported that patients with arterial hypertension and coronary insufficiency rated the exertion high in relation to heart rate as compared with controls

In the present investigation different groups of patients were compared with healthy subjects of comparable age in order to further elucidate these differences At the same time different methods of estimating the physical working capacity have been compared

## MATERIAL

Groups of patients with the vasoregulatory asthenia syndrome (8) coronary heart disease and arterial hypertension were selected from about 7000 patients who were examined during a three year period for diagnostic purposes with ECG at rest, during and after exercise at the Department of Clinical Physiology University Hospital Umeå Consecutively examined patients who fulfilled the criteria to be described below were included in the study All patients with digitalis medication signs of valvular heart disease and with ECG changes or history typical of previous heart infarction were excluded. Groups of healthy age-compatible control subjects were included in the study for comparison Some general characteristics of the groups are given in Table I The patient groups are to a great extent identical with those included in a study on the effects of hyperventilation on ECG (6)

[1] A report of this paper was given at the Umeå Medical Association in 1967

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Table III *Mean heart rate at various rating values* ($\bar{X}$) *and mean differences* ($\bar{D}$) *between groups of males and females of equal age* ($\bar{D}$)

| Group (age range) | Sex | | Rating value 9 | 13 | 17 |
|---|---|---|---|---|---|
| $N_{III}$ (18–20) | ♂ | $n$ | 16 | 29 | |
| | | $\bar{X}$ | 137 | 159 | |
| | | $S$ | 18 | 20 | |
| | ♀ | $n$ | 14 | 12 | |
| | | $\bar{X}$ | 134 | 162 | |
| | | $S$ | 19 | 14 | |
| | | $\bar{D}$ (♂–♀) | 3 | −3 | |
| $N_{IV}$ (20–24) | ♂ | $n$ | 24 | 27 | 19 |
| | | $\bar{X}$ | 121 | 147 | 172 |
| | | $S$ | 12 | 17 | 10 |
| | ♀ | $n$ | 18 | 20 | 13 |
| | | $\bar{X}$ | 132 | 154 | 178 |
| | | $S_x$ | 13 | 15 | 8 |
| | | $\bar{D}$ (♂–♀) | −11* | −7 | −6 |
| $N_{II}$ (50–59) | ♂ | $n$ | 15 | 25 | |
| | | $\bar{X}$ | 102 | 126 | |
| | | $S_x$ | 16 | 17 | |
| | ♀ | $n$ | 7 | 8 | |
| | | $\bar{X}$ | 107 | 134 | |
| | | $S_x$ | 19 | 17 | |
| | | $\bar{D}$ (♂–♀) | −5 | −8 | |

In this and the following tables the probability ($P$) that differences are caused by random factors is denoted as follows * $= 0.05 > P > 0.01$ ** $= 0.01 > P > 0.001$ *** $= P < 0.001$ Differences without asterisks $= P > 0.05$ Other symbols as in Tables I and II

the work was interrupted Such rating values have not been used in this analysis

*PWC$_F$* was estimated as described by Borg and Linderholm (5) by using a heart rate reference level (HRL) variable with age between 20 and 80 years of age according to the equation $HRL_{80\%} = 170 - [2(A - 20)/3]$ where A is the age in years and $HRL_{80}$ corresponds to about 80 of the maximal possible increase in heart rate from the resting level The submaximal working capacity at this heart frequency level was $PWC_F$

*PWC$_m$* The maximal work intensity performed $PWC_m$ was taken as the heaviest load at which the subject actually worked for 6 min If the subject was able to work on a higher load for less than 6 min a fraction of the stepwise increase in work load corresponding to a fraction of the 6 min period in which the subject was able to continue work on this higher load was added (cf ref 14) No attempt was made to get objective evidence of maximal performance in terms of high blood lactate concentrations etc and $PWC_m$ is not regarded as being equivalent to measure of maximal performance where such methods were employed (cf also Discussion)

*Methodological error of the PWC measures*

An estimate of the error of the PWC measures can be obtained from *intra test correlations* of heart rates and rating values of perceived exertion

By using heart rates from the work loads 300 and 900 kpm/min to obtain one estimate of $PWC_{170}$ and the heart rates from 600 and 1200 kpm/min to obtain another estimate of $PWC_{170}$ from work tests which included these four work loads the correlation between two $PWC_{170}$ values obtined from different parts of the same work tests was calculated in a group of 54 healthy subjects The correlation coefficient was found to be 0 84 and the reliability coefficent was estimated at 0 91 (after correction according to Spearman Brown s formula) (7)

In a similar way two $PWC_R$ values were estimated from rating values of perceived exertion at work loads 300 and 900 kpm/min and from ratings at 600 and 1200 kpm/min The correlation between the two PWC values was calculated for the same group of 54 subjects The correlation coefficient was 0 85 and the reliability coefficient was estimated at 0 92 (after correction according to Spearman Brown s formula)

The intra test correlations obtained for the two different kinds of measure of PWC indicate a low methodological error This error tends to decrease at a high reference level of heart frequency or rating (vide infra) and therefore the intra test correlation for $PWC_{170}$ and for $PWC_R$ should be at least 0 90 PWC or PWC was about 1 00 ± 200 (mean ± S D) kpm/min Then the error of a single determination ($s$) of PWC according to the formula $s = s\sqrt{1-r}$ (where $s$ is the S D in the group) should be $s = 200 \times 0.3 = 60$ kpm/min i e about 6 of the mean value

The error of the PWC measures can also be estimated from *repeated examinations* of the same individuals Using this approach the overall error in the measures of PWC was estimated from double determinations made with an interval of 2–4 weeks A comparatively long period between the tests was chosen in order to minimize the effect of memory on the rating of perceived exertion Patients with various heart and other diseases but with comparatively well preserved physical working capacity and healthy subjects were used for this study The results appear in Table II The measures based on observations made on a relatively high work load $PWC_{170}$ and $PWC_R$ have a smaller error than those based on observations obtained at relatively low work loads PWC and $PWC_R$ The error of the determination of the PWC measures based on heart rate seems to be sligthly smaller in the patient group than those based on observations made on the rating of perceived exertion The error of the $PWC_r$ is larger than that found in normal subjects when double determinations were made with an interval of 1 2 d ys (10) but of the same magnitude as that reported by Bevegård et al (2) when examining patients with heart disease with an interval between double determinations comparable to that used by us

## RESULTS

*The relationship between heart rate and rating of perceived exertion in the groups of patients and healthy controls*

Healthy male and female controls of three age groups 18–20 20–24 and 50–69 years of age

Table IV *Mean heart rate of control and patient groups and mean differences in heart rate between groups at rating values $R_9$ (rather light) $R_{13}$ (rather laborious) and $R_{17}$ (very laborious)*

Figures within brackets give the number of subjects In case of differences the figures within brackets represent the number of subjects in the last group Symbols as in Tabels I and III

| Group | Rating value 9 | | 13 | | 17 | |
|---|---|---|---|---|---|---|
| | *Mean value heart rate of healthy cont ol g oups* | | | | | |
| $N_I$ | 121 | (84) | 149 | (90) | 172 | (49) |
| $N_{II}$ | 103 | (20) | 1 8 | (3?) | 152 | (13) |
| LN | 101 | (30) | 129 | (35) | 157 | (?1) |
| | *Mean d fference in hea t rate bet een groups* | | | | | |
| $N_I$–VA | −24 4 | (10) | −16 1 | ( 0) | −7 1 | (12) |
| $N_{II}$–$C_I$ | −4 2 | ( 0) | 4 8 | ( 6) | 15 3 | ( 0) |
| $N_{II}$–$C_{II}$ | −3 3 | (7) | 11 0 | (10) | 12 2 | (9) |
| LN–LC | 7 7 | (6) | 22 4 | (7) | 21 0 | (5) |
| $(N_{II}+LN)-(C_I+C_{II}+LC)$ | −2 8 | (33) | 9 4 | (43) | 17 3 | (34) |
| $N_{II}$–$H_I$ | −9 7 | (19) | −1 7 | (30) | 5 4 | (24) |
| $N_{II}$–$H_{II}$ | −1?1 | (23) | −0 7 | (31) | 1 1 | (21) |
| LN LP | −3 8 | (10) | 5 9 | (9) | 10 8 | (6) |
| $N_{II}$–$N_I$ | −18 1 | (84) | −20 4 | (90) | −?0 1 | (49) |
| $N_{II}$–LN | 2 3 | (30) | −0 7 | (35) | −4 3 | ( 1) |

were compared with regard to the heart rate at given rating values of perceived exertion (Table III) The females had on an average a sligthly higher heart rate at equal rating The difference was not statistically significant except at low rating $R_9$ in the group 20–24 years of age ($P < 0.05$) Owing to the small difference between men and women we treated them together when comparing the relationship between heart rate and rating of perceived exertion in the patient and control groups

Figs 1–3 show the relationship between heart frequency and perceived exertion in some different patient groups compared with control groups The differences in heart rate at given rating values of perceived exertion between control and patient groups and their statistical significance are given in Table IV Table V gives the slopes

Table V *Mean increase in heart rate for increase in rating value from 9 to 13 Δ (heart rate)$_{R9-13}$ and from 13 to 17 Δ (heart rate)$_{R13-17}$ and mean differences in these mean increases in heart rate between groups*

Figures within brackets give the number of subjects In case of differences the figures within brackets represent the number of subjects in the last group Symbols as in Tables I and III

| Group | Δ (heart rate)$_{R9-13}$ | | Δ (heart rate)$_{R13-17}$ | |
|---|---|---|---|---|
| | *Mean increase in heart rate* | | | |
| $N_I$ | 27 7 | (83) | ?8 9 | (49) |
| $N_{II}$ | 22 2 | (19) | 31 1 | (13) |
| LN | 32 3 | (28) | ?2 3 | (?1) |
| | *Mean d ff nce* | | | |
| $N_I$–VA | 4 6 | (9) | 8 6 | (12) |
| $N_{II}$–$C_I$ | 5 5 | (15) | 14 7 | (16) |
| $N_{II}$–$C_{II}$ | 3 3 | (7) | 4 0 | (9) |
| LN–LC | 14 8 | (6) | −5 5 | (5) |
| $(N_{II}+LN)-(C_I+C_{II}+LC)$ | 10 8 | (28) | 8 6 | (30) |
| $N_{II}$–$H_I$ | 2 6 | (19) | 8 5 | (18) |
| $N_{II}$–$H_{II}$ | 1 7 | (23) | 6 9 | (21) |
| LN LP | 14 1 | (9) | 5 8 | (6) |
| $N_{II}$–$N_I$ | −5 5 | (83) | [illegible] | (49) |
| $N_{II}$–LN | −10 1 | (28) | −1 2 | (21) |

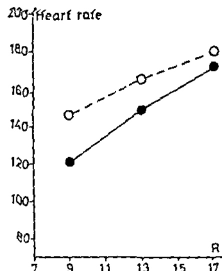



*Fig 1* The relationship between heart rate and perceived exertion (*R*) in a control group ($N_I$) ●—● and a group of patients with vasoregulatory asthenia (VA) O--O including males and females

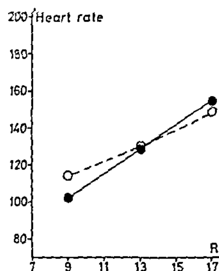



*Fig 3* The relationship between heart rate and percei exertion (*R*) in a control group ($N_{II}$ – LN) ●—● a two groups of patients with arterial hypertension ($H_I$ a $H_{II}$) O--O including males and females

i e the increase in heart rate for increase in the rating value from 9 to 13 Δ(heart rate)$_{R9\ 13}$ and from the rating 13 to 17 Δ(heart rate)$_{R13\ 17}$ in various groups as well as differences in slopes between control and patient groups

*Vasoregulatory asthenia* Fig 1 and Table IV demonstrate differences between the VA group and the control group at the rating values 9 and 13 but not at high rating values of perceived exe tion ($R_{17}$) The slope Δ(heart rate)$_{R13\ 17}$ of t VA groups was statistically significantly smal than that of the controls (Table V) There we only slight and statistically insignificant differenc in heart rate at given rating values and in slop between VA patients with a $PWC_{170}$ less tha the mean of the group and those with a $PWC_{170}$ above the mean

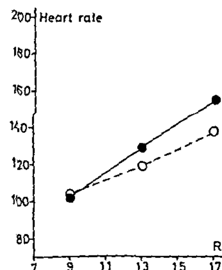



*Fig 2* The relationship between heart rate and perceived exertion (*R*) in a control group (N – LN) ●—● and three groups of patients with coronary insuficiency ($C_I$ $C_{II}$ and LC) O--O including males and females

*The coronary insufficiency* group with a lo physical working capacity $C_I$ differed statisticall significantly in heart rate at the rating value 1 from the control group of comparable age (Tab IV) No such differences were present at the ra ing values 9 and 13 or in the coronary insuff ciency group with an ordinary physical workin capacity $C_{II}$ The slope Δ(heart rate)$_{R13\ 17}$ of the group $C_I$ (but not group $C_{II}$) was statisticall significantly smaller than that of the contro (Table V)

In the group of lumber workers with corona insufficiency group LC the heart rate at the ra ing values 13 and 17 as well as the slope Δ(hea rate)$_{R\ 13\ 17}$ was smaller than that of the contr group of healthy lumber workers group LN (Tables IV and V) In these respects the lumbe workers with coronary insufficiency differ fro the group of lumber workers with pains in kne joints and back group LP which did not diffe

Table VI *Mean values ($\bar{x}$) of maximal recorded heart rate and various measures of physical working capacity in the different patient groups and in groups of healthy controls*

Mean values ($x$) and the number of subjects ($n$) from which the means were calculated Symbols as in Tables I and II

| | | Max heart rate | | $PWC_{130}$ | | $PWC_{170}$ | | $PWC_{R13}$ | | $PWC_{R17}$ | | $PWC_{P}$ | | $PWC_{max}$ | | $PWC_{130}/PWC_{R13}$ | | $PWC_{P}/PWC_{?}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | Sex | $n$ | $x$ | $n$ | $x$ | $n$ | $x$ | $n$ | $\bar{x}$ | $n$ | $x$ | $n$ | $x$ | $n$ | $x$ | $n$ | $x$ | $n$ | $x$ |
| VA | ♂ | 11 | 188 | 11 | 247 | 11 | 573 | 10 | 485 | 8 | 789 | 11 | 521 | 11 | 745 | 10 | 0 59 | 8 | 0 69 |
| | ♀ | 11 | 18 | 10 | 88 | 11 | 358 | 11 | 328 | 10 | 501 | 11 | 292 | 11 | 467 | 10 | 0 32 | 10 | 0 60 |
| $C_I$ | ♂ | 24 | 135 | 17 | 488 | 3 | 770 | 20 | 419 | 13 | 558 | 17 | 635 | 24 | 513 | 15 | 1 15 | 10 | 1 03 |
| | ♀ | 8 | 149 | 5 | 291 | [illegible] | 305 | 8 | 317 | 3 | 457 | 7 | 476 | 8 | 404 | 4 | 0 82 | 3 | 0 76 |
| $C_{II}$ | ♂ | 8 | 145 | 8 | 773 | 3 | 995 | 8 | 597 | 8 | 909 | 8 | 971 | 8 | 906 | 8 | 1 41 | 8 | 1 09 |
| | ♀ | 2 | 167 | 2 | 470 | 2 | 655 | 2 | 385 | 2 | 555 | 2 | 545 | 2 | 617 | 2 | 1 31 | [illegible] | 1 05 |
| $H_I$ | ♂ | 13 | 151 | 10 | 499 | 4 | 686 | 11 | 486 | 5 | 671 | 10 | 637 | 13 | 592 | 10 | 1 00 | 5 | 0 87 |
| | ♀ | 2? | 151 | 19 | 309 | 8 | 468 | 17 | 317 | 14 | 418 | 19 | 401 | 22 | 408 | 16 | 0 99 | 13 | 0 90 |
| $H_{II}$ | ♂ | 14 | 151 | 14 | 686 | 7 | 1039 | 14 | 677 | 11 | 936 | 14 | 846 | 14 | 945 | 14 | 1 04 | 11 | 0 85 |
| | ♀ | 16 | 164 | 16 | 407 | 12 | 654 | 16 | 39[illegible] | 14 | 584 | 16 | 512 | 16 | 610 | 16 | 1 0 | 14 | 0 90 |
| $N_I$ | ♂ | 71 | 179 | 71 | 720 | 71 | 1188 | 70 | 905 | 59 | 1 66 | 71 | 1110 | 71 | 1 91 | 70 | 0 81 | 59 | 0 90 |
| | ♀ | 20 | 184 | 20 | 388 | 20 | 670 | 20 | 564 | 18 | 759 | 20 | 648 | 20 | 760 | 20 | 0 70 | 18 | 0 86 |
| $N_{II}$ | ♂ | 25 | 157 | 24 | 697 | 18 | 1088 | 25 | 647 | [illegible]1 | 984 | 24 | 847 | [illegible]5 | 951 | 24 | 1 1[illegible] | 21 | 0 89 |
| | ♀ | 8 | 155 | 8 | 488 | 2 | 760 | 8 | 527 | 4 | 715 | 8 | 569 | 8 | 634 | 8 | 1 00 | 4 | 0 80 |
| LN | ♂ | 35 | 16? | 35 | 878 | 23 | 1194 | 35 | 896 | 27 | 1 10 | 34 | 1062 | 35 | 1110 | 35 | 1 03 | 26 | 0 89 |
| LC | ♂ | 7 | 147 | 7 | 900 | 1 | 1470 | 7 | 643 | 5 | 10 0 | 7 | 1116 | 7 | 10?1 | 7 | 1 66 | 5 | 1 18 |
| LP | ♂ | 10 | 146 | 9 | 767 | 2 | 1110 | 9 | 708 | 7 | 1071 | 7 | 877 | 10 | 915 | 9 | 1 15 | 5 | 0 84 |

statistically from the control group of lumber workers

By adding $N_{II}$ and LN to form a larger control group and comparing this group with a coronary insufficiency group obtained by adding $C_I$ $C_{II}$ and LC highly significant differences between the control group and the coronary insufficiency group were obtained in the relationship between heart rate and rating value (see Table IV and Fig 2)

The groups with *arterial hypertension* deviated from the controls in a similar way to the VA group but less markedly A statistically significant difference in heart rate ($P < 0\ 05$) was obtained at the rating value 9 ( rather light work) between group $N_{II}$ and the groups $H_I$ and $H_{II}$ (Table IV) There was a slight difference in slope between group $H_I$ and group $N_{II}$ (Table V cf also Fig 3)

*Control groups* There were obvious differences between the young and old subjects as shown by control groups $N_I$ *and* $N_{II}$ *It should be noted* that the difference between the VA group and group $N_I$ in heart rate at a given rating value is not similar to that between group $N_I$ and group $N_{II}$ This is also shown by the differences in slopes $\Delta$(heart rate)$_{P9-13}$ and $\Delta$(heart rate)$_{P13-1}$ between ($N_I$ − VA) and ($N_{II}$ − $N_I$) see Table V

*Physical working capacity estimated from heart rate rating of perceived exertion and work performed ($PWC_{m\ x}$)*

Some results of the various methods of estimating physical working capacity in the patient and control groups are given in Table VI With the restriction on extrapolation in the work load–heart rate or work load–rating value diagrams (see Methods) *applied in the present investigation all* measures of PWC were not obtained in all subjects Particularly among the patients with coronary insufficiency and arterial hypertension $PWC_{1\ 0}$ was obtained from a limited number of subjects due to the fact that the heart rate on the highest work load was often less than 150 beats/min In some cases it was less than 110 beats/min and then $PWC_{1\ 0}$ was not obtained Several subjects did not rate the perceived exertion so high ($R_{1}$) after more than 4 min work on the highest load that $PWC_{P1}$ *could be estimated*

According to earlier described changes in the relation between heart rate and rating of perceived exertion with age (5) the PWC values calculated from the heart rate as well as $PWC_{ma}$ were

Table VII *Mean differences between groups in the ratios $PWC_{130}/PWC_{R13}$ and $PWC_P/PWC_{R17}$*

The groups include males and females and correspond to those given in Table VI

| Group | $PWC_{130}$/ $PWC_{R13}$ | $PWC_P$/ $PWC_{R17}$ |
|---|---|---|
| $N_I$–VA | 0 34* | 0 25*** |
| $N_{II}$–$C_I$ | 0 01 | −0 10 |
| $N_{II}$–$C_{II}$ | −0 30* | −0 21* |
| LN–LC | −0 63 | −0 29 |
| ($N_{II}$+LN)–($C_I$+$C_{II}$+LC) | −0 22 | −0 17** |
| $N_{II}$–$H_I$ | 0 09 | −0 02 |
| $N_{II}$–$H_{II}$ | −0 03 | −0 01 |
| LN–LP | −0 12 | 0 06 |
| $N_{II}$–$N_I$ | 0 30 * | −0 02 |
| $N_{II}$–LN | 0 06 | −0 02 |

relatively low in relation to those obtained from the rating of perceived exertion in the control group $N_I$ (20–39 years of age) when compared with the control group $N_{II}$ (50–69 years of age) Still lower PWC values estimated from the heart rate in relation to those obtained from the rating of perceived exertion and $PWC_{max}$ were found in the VA group

The patients of groups $C_I$ and $H_I$ who had low maximal physical performance PWC $_a$ also had low $PWC_{1\ 0}$ and $PWC_{R13}$ compared with the control group $N_{II}$ *Patients of groups $C_{II}$ and $H_{II}$* had approximately similar mean PWC values to the controls $PWC_1$ compared fairly well with $PWC_{R1}$ and $PWC_{max}$ in all except the VA group in which $PWC_1$ was relatively low

*The ratios $PWC_{130}/PWC_{T1}$ and $PWC_P/PWC_{R1}$*

Out of several possible ratios between the measures of physical working capacity the ratios $PWC_{1\ 0}/PWC_{R13}$ and $PWC_P/PWC_{R17}$ were found to be particularly interesting These ratios were found to be fairly equal in male and female groups (Table VI) and therefore in a comparison of these ratios between groups males and females were treated together (Table VII)

The ratio $PWC_{130}/PWC_{P13}$ was higher in group $N_{II}$ than in $N_I$ while the ratio $PWC_P/PWC_{P17}$ seems to be quite independent of age (Tables VI and VII)

In the VA group the two ratios $PWC_{1\ 0}$/ $PWC_{R13}$ and $PWC_P/PWC_{R1}$ were low while they were high in the groups of coronary insufficiency when compared with age compatible control groups Patients with hypertension did not d from healthy controls nor from the group of lumber workers with pain in back and legs (Table VI and VII)

## DISCUSSION

The results show that there is a difference between the controls and the patient groups The VA patients have a high heart rate in relation to the rating of perceived exertion particularly at the low work loads They do not rate the exertion as high as do the controls at the same comparatively low heart rates The patients with arterial hypertension deviate from their controls in a similar way though less markedly a result which deviates somewhat from that of the preliminary study (4) On the other hand the patients with coronary insufficiency especially those with a low work tolerance have a low heart rate in relation to their rating of exertion particularly at high rating values

All patient groups are similar in the respect that the mean value for their increase in heart rate in relation to increase in rating of subjective exertion is smaller than in the control groups Stated in another way the patients perceived exertion increases more rapidly with a given increase in heart rate than that of controls

As a consequence of the deviation of some patient groups from normal relationships between heart rate and rating of perceived exertion during exercise the relationships between the different measures of PWC used in this study also deviate from those found in healthy controls of comparable age In the VA group the measures of PWC based on heart rate $PWC_{130}$ and $PWC_{1\ 0}$ are thus comparatively low in relation to those based on rating of perceived exertion $PWC_{R13}$ and $PWC_{R1}$ while the opposite though less markedly so is true of patients with coronary insufficiency Young and old control groups differ to some extent in a similar way to VA and coronary insufficiency patient groups

Some ratios between the measures of physical *working capacity $PWC_{130}/PWC_{R13}$ and $PWC_P$/* $PWC_{R1}$ are useful for demonstrating these differences There do not seem to be marked sex differences in these ratios and *the ratio $PWC_P$/* $PWC_{R17}$ seems to be independent of age The ratio $PWC_{130}/PWC_{R13}$ can be used in the cas

of patients who do not reach high heart rates during exercise Both these ratios are high in the VA group and low in the coronary insufficiency groups Instead of $PWC_{130}$ another $PWC_P$ value where P refers to lower pulse or heart rates than those used in this study might be useful

The $PWC_{max}$ (cf Methods) may be regarded as an index of the behaviour of the subject in an experimental situation in which the subject is expected to perform physical exercise as well as possible It is usually not possible to expect maximal performance from the patients but it is likely that most of them worked fairly close to a real maximal performance It is interesting to note that in those groups in which the $PWC_{m}$ was low also the $PWC_{130}$ and $PWC_{R13}$ were low The last mentioned submaximal measures of physical working capacity were in most cases based mainly on observations obtained at a level of work load when subjective complaints had not become pronounced In this connection it is interesting that a group of lumber workers ($n = 10$) who complained of pains in the knee joints and in the back rated the perceived exertion in relation to heart rate in a similar way to healthy workers

The patients with vasoregulatory asthenia have a high heart frequency in relation to the rated value of perceived exertion They are known to have a hyperkinetic circulation but normal blood pressures in the systemic and lesser circulation at rest as well as during exercise The blood lactate during exercise increases less in relation to heart rate than in ordinary control groups and in patients with heart disease such as mitral stenosis (9) This may be one explanation for the perception of low exertion in relation to heart rate in VA patients as the perception of exertion and blood lactate concentration during exercise seem to be closely connected in normal subjects (3)

In the same way a comparatively high blood lactate concentration may be one possible explanation of the fact that patients with coronary insufficiency rate the exertion high in relation to the heart rate Patients with coronary insufficiency and a hypokinetic circulation (cf 11) might be expected to have comparatively high blood concentration of lactate in relation to heart rate during exercise as is the case in patients with hypokinetic circulation due to mitral stenosis (9)

It may be reasonable to assume that sensations related to the disturbed hemodynamic conditions in coronary heart disease should contribute to the results It is known that these patients usually have a hypokinetic circulation with low cardiac output and stroke volume during exercise and a high blood pressure in the lesser circulation (11)

However the patients with arterial hypertension who have similar hemodynamic changes to patients with coronary heart disease (15) did not rate the exertion high in relation to heart rate Nor was there a difference between group $H_I$ in which the hemodynamic change presumably was pronounced and group $H_{II}$ with less pronounced hemodynamic disturbance Therefore sensations caused by this type of hemodynamic disturbance do not seem to have much influence on the relationship between rating of perceived exertion and heart rate Nor should anginal pain be of much importance as the work was usually interrupted soon after anginal pain appeared

The signs and symptoms in patients with the vasoregulatory asthenia syndrome and patients with coronary heart disease may sometimes be quite similar (8) The difference in the rating of perceived exertion in relation to heart rate during exercise found between these groups of patients is therefore of differential diagnostic value


## ACKNOWLEDGEMENT

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## REFERENCES


1 Arvedson O Furberg C & Linderholm H Effect of a ganglionic blocking agent (chlorisondamine) on electrocardiogram physical working capacity and hemodynamics in patients with vasoregulatory asthenia Acta med scand Suppl 47? 36 1967

2 Bevegård S & Jonsson B Reproducerbarhet av arbetsprov i ett patientmaterial Riksstamman (Meeting of the Swedish Medical Association) Stockholm 1966

3 Borg G Physical performance and perceived exertion Thesis Lund 196

4 Borg G & Linderholm, H Upplevelse av fysiskt arbete förhållande till arbetsprestation hos vissa patientgrupper Riksstamman (Meeting of the Swedish Medical Association) Stockholm 1960

5 — Perceived exertion and pulse rate during graded exercise in various age groups Acta med scand Suppl 47? 194 1967

6 Furberg C & Linderholm H Effect of hyperventilation on ECG in patients with circulatory disturbances Acta med scand 18? 167 1969

7 Garret H E Statistics in psychology and education 5th ed Longmans Green and Co New York 1958
8 Holmgren A Jonsson B Levander M Linderholm H Sjostrand T & Strom G Low physical working capacity in suspected heart cases due to inadequate adjustment of peripheral blood flow (vasoregulatory asthenia) Acta med scand 158 413 1957
9 Holmgren A & Strom G Blood lactate concentration in relation to absolute and relative work load in normal men and in mitral stenosis arterial septal defect and vasoregulatory asthenia Acta med scand 163 185 1959
10 Linderholm H Unpublished observations
11 Malmborg R A clinical and hemodynamic analysis of factors limiting the cardiac performance in patients with coronary heart disease Thesis Stockholm 1964
12 Sandberg L Studies on electrocardiographic changes during exercise Acta med scand Suppl 356 1961
13 Sjostrand T Changes in the respiratory organs of workmen at an ore smelting works Acta med scand Suppl 196 687 1947
14 Strandell T Circulatory studies on healthy old men with special reference to the limitation of the maximal physical working capacity Acta med scand Suppl 414 1964
15 Varnauskas E Studies in hypertensive cardiovascular disease with special reference to cardiac function Thesis Stockholm 1955
16 Wahlund H Determination of physical working capacity Acta med scand Suppl 215 1948

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# OCCURRENCE OF INTERSTITIAL NEPHRITIS IN ACUTE RENAL FAILURE

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*Abstract* Renal biopsies of 63 patients with acute oliguric renal failure have been studied Interstitial cellular infiltration and oedema occurred in 76% of the patients Follow up biopsies on 18 patients showed that chronic interstitial changes are frequent at least when the acute renal failure is due to a nephrotoxic agent

The concept of acute interstitial nephritis has been discussed for instance by Zollinger (9) who considers it a clinico-pathological entity with varying aetiology Most of the proposed aetiological factors potentially give rise to acute renal failure According to previous reports dealing with the histopathology of the kidney in acute renal failure (acute tubular necrosis lower nephron nephrosis shock kidney etc) interstitial cellular infiltration and oedema occur in 30–100% of the cases (1 7 9) It has also been suggested (9) that acute oliguric renal failure showing acute interstitial nephritis may later become what is called chronic interstitial nephritis

In our series of renal biopsies on patients with acute renal failure we repeatedly found interstitial changes To find out the cause of this high incidence we took into consideration the various aetiological factors the time of renal biopsy in relation to onset of acute renal failure and the patient s age

To check the suggested development of acute interstitial nephritis into chronic interstitial nephritis we followed up 18 patients by repeated renal biopsies

## MATERIAL AND METHODS

The material consists of 63 patients in whom renal biopsy was performed because of acute renal failure presenting anuria or oliguria Patients with exacerbation of a chronic renal disease as well as those with acute or chronic glomerulonephritis, collagen diseases, obstructive uropathy and pyelonephritis were excluded The patients were selected consecutively among all patients who had a renal biopsy during 196 –1968 They fall into different aetiological groups, as presented in Table I The age distribution of the patients is shown in Table II The mean age of all patients was 36 ? years

Biopsies were performed on the third to the 36th day after onset of anuria or oliguria Forty five patients had only one renal biopsy Eighteen patients had one or more additional renal biopsies performed one to 233 weeks after the first Table III shows the distribution of the patients according to the day of the first renal biopsy

The biopsy specimens were fixed in 10% neutral formaldehyde embedded in paraffin wax and sectioned at 3–5 μ The following staining methods were used H-E PAS H-vG and Gomori s silver methenamine stain. The sections were studied for the presence of tubular necrosis and atrophy of the tubular elements interstitial oedema, interstitial cellular components and glomerular and vascular changes

## RESULTS

### *First Biopsies*

*Tubular necrosis* was present in only one patient This was a case of arsenic intoxication In this patient a new biopsy performed 4 1/ months later showed normal tubuli fibrosis of the interstitium round cell infiltration vascular changes and glomerular hyalinization The patient was 41 years old and had no hypertension Functional restitution was observed at the second biopsy

*Tubular atrophy* was a significant finding in only two biopsies One patient 48 years old had acute phenacetin intoxication and the other aged 18 years developed acute renal failure after an orthopaedic operation

*Interstitial oedema* occurred in 32 of the first

Table I. *Aetiology of acute renal failure in 63 patients*

| | No. of pats. | |
|---|---|---|
| Intoxication | | |
| Ethylene glycol | 10 | |
| Ethanol | 3 | |
| CCl | 1 | |
| Phenacetin | 1 | |
| Aspirin | 1 | |
| Arsenic | 1 | |
| AgCl suspension | 1 | |
| PAS (p-amino salicylic acid) | 1 | |
| Other | 3 | 22 |
| Obstetric complications | | |
| Abortions | 7 | |
| Portal contraction | 4 | |
| Ablatio placentae | 1 | |
| Eclampsia | 2 | |
| Other | 2 | 16 |
| Infectious diseases | | |
| Epidemic nephropathy | 6 | |
| Acute appendicitis | 2 | |
| Acute respiratory infection | 4 | |
| Colonic diverticulitis | 1 | 13 |
| Oligaemia, shock | | 6 |
| Haemolysis, myolysis | | 3 |
| Other | | 3 |

Table II. *Age distribution of 63 patients with acute renal failure*

| Age (y) | No. of pats. |
|---|---|
| 1–20 | 10 |
| 21–30 | 14 |
| 31–40 | 16 |
| 41–50 | 7 |
| 51–60 | 12 |
| 61– | 4 |

Table III. *Day of first biopsy after onset of acute renal failure in 63 patients*

| Days | No. of pats. |
|---|---|
| 1–7 | 8 |
| 8–14 | 20 |
| 15–21 | 21 |
| 22–36 | 14 |

biopsies. The patients showing this alteration are divided into aetiological groups in Table IV. Those showing no interstitial oedema are presented for comparison. The time of biopsy in relation to the onset of acute renal failure in the groups with and without interstitial oedema is plotted in Table V.

The age distribution of the patients with and without interstitial oedema is shown in Table VI.

*Interstitial cellular infiltration.* Cellular infiltration in the renal interstitium occurred in 43 of the first biopsies. In 27 instances the infiltration contained granulocytes, in 37 instances lymphocytes and plasma cells. In 23 biopsies both types of cells occurred. Twenty patients lacked all these cellular alterations in the first biopsies. In 27 patients interstitial cellular infiltration and oedema were co-existent. The aetiology of the acute renal failure in the patients with and without interstitial cellular infiltration is shown in Table IV. The time of biopsy in relation to the onset of acute renal failure in the groups with and without interstitial cellular infiltration appears in Table V. Table VI shows the age distribution of the patients with and without interstitial cellular infiltration.

*Interstitial fibrosis* occurred in seven of the first biopsies. These patients were 18, 33, 43, 4?, 51, 61 and 62 years old.

*Hyalinized glomeruli.* Glomerular hyalinization

Table IV. *Occurrence of interstitial cellular infiltration and oedema in renal biopsies of 63 patients with acute renal failure*

| | Interstitial oedema | | Interstitial infiltration | | |
|---|---|---|---|---|---|
| Aetiology | Present | Not present | Present | Not present | Total |
| Intoxication | 14 | 8 | 17 | 5 | 22 |
| Obstetric | 7 | 9 | 12 | 4 | 16 |
| Infectious | 7 | 6 | 7 | 6 | 13 |
| Oligaemia | 1 | 5 | 3 | 3 | 6 |
| Haemolysis, myolysis | 1 | 2 | 1 | 2 | 3 |
| Other | 2 | 1 | 3 | 0 | 3 |

Table V *Occurrence of interstitial oedema and cellular infiltration in renal biopsies from 63 patients with acute renal failure divided into groups according to the time of biopsy*

| Day of biopsy after onset of anuria | Interstitial oedema | | Interstitial infiltration | | |
|---|---|---|---|---|---|
| | Present | Not present | Present | Not present | Total |
| 1-7 | 4 | 4 | 5 | 3 | 8 |
| 8 14 | 12 | 8 | 13 | 7 | 20 |
| 15-21 | 14 | 7 | 18 | 3 | 21 |
| 36 | 2 | 12 | 7 | 7 | 14 |

Table VI *Occurrence of interstitial oedema and cellular infiltration in renal biopsies of 63 patients with acute renal failure divided into age groups*

| Age (y) | Interstitial oedema | | Interstitial infiltration | | |
|---|---|---|---|---|---|
| | Present | Not present | Present | Not present | Total |
| 20 | 4 | 6 | 7 | 3 | 10 |
| 21 30 | 9 | 5 | 10 | 4 | 14 |
| 31-40 | 7 | 9 | 9 | 7 | 16 |
| 41 50 | 3 | 4 | 3 | 4 | 7 |
| 51- | 10 | 6 | 13 | 3 | 16 |

occurred in nine of the first biopsies The age of these patients ranged from 48 to 68 years their mean age being 57 4 years

*Vascular changes* appeared in seven patients Six of them had hyalinized glomeruli The age of these patients varied between 33 and 68 years with a mean age of 54 7 years

*Follow up Biopsies*

In 18 patients two or more biopsies were performed Interest was focused on the occurrence of interstitial changes The presence of interstitial oedema and infiltration with granulocytes and lymphocytes was defined as acute interstitial nephritis Interstitial fibrosis and infiltration with lym

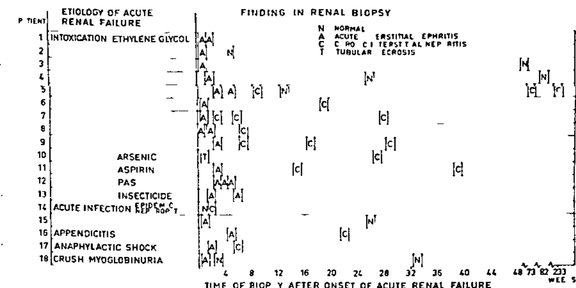



*Fig 1* Follow up study of renal biopsies in 18 patients



Fig. 2. Renal biopsy 14 days after onset of anuria. Patient no. 17 (cf. Fig. 1). The biopsy shows interstitial oedema and infiltration of inflammatory cells, among them polymorphonuclear leukocytes. Haematoxylin-eosin. ×2[illegible]

processes and plasma cells such was called chronic interstitial nephritis. The findings in these biopsies appear in Fig. 1 which also gives the diagnosis of the patients.

Figs. 2 and 3 show the main histopathological findings in one patient (no. 17) at 14 and 50 days after onset of anuria.

## DISCUSSION

One of the characteristics of the present series that accorded with previous reports (1, 2) was the almost complete absence of tubular necrosis. Only the one patient with arsenic intoxication exhibited this lesion, which is found elsewhere in conditions due to so-called nephrotoxic agents (6).

Another characteristic feature of the present series was the high frequency of infiltration and oedema of interstitial tissue. This lesion, designated interstitial nephritis, occurred in 4[illegible] of the 63 cases (75%). In Brun and Munck's series interstitial changes only occurred in one third of the cases. Price and Palmer (7) found interstitial fibrosis and cellular infiltration in seven of eight patients who were biopsied 5 weeks to [illegible] years after the acute episode.

The question arises whether there exists any cause for the variations in the frequency of interstitial nephritis in the various series. From the present series one gains the impression that interstitial changes, though present in almost all aetiological groups, were most characteristic of intoxications and obstetric complications. Five of the seven positive cases of Price and Palmer (7) belonged to this group. In the series of Brun and



Fig. 3. Renal biopsy 5[illegible] days after onset of anuria. Patient no. 17 (cf. Fig. 1). The biopsy showed [illegible] fibrosis [illegible] distribution of cellular infiltration, most of lymphocytes and plasma cells, and [illegible]. Haematoxylin-eosin. ×1[illegible]

Munck (1) the aetiology of acute renal failure was not given in the individual cases with interstitial changes but cases both with intoxications and obstetric complications were less frequent than in the present material The small number of cases in each group in the present series precludes any definite conclusion but it seems possible that at least acute renal failure resulting from intoxications and from obstetric events often presents with acute interstitial changes a fact pointed out by Richet et al (8) and Oliver et al (6) Interstitial cellular infiltration and oedema could not be related to the patient s age The occurrence of interstitial fibrosis vascular changes and hyalinized glomeruli seemed to be related to advanced age Oliver (5) and Oliver et al (6) in a chronological description of acute tubular necrosis pointed out that interstitial oedema appears at a very early stage Later on it is followed by interstitial cellular infiltration When renal function begins to be restituted both oedema and cellular infiltration regress In our series interstitial oedema was frequent in cases biopsied up to the 20th day after onset of anuria while even later interstitial cellular infiltration appeared relatively often

It is the general view that recovery from acute tubular necrosis is consistent with complete morphological and functional healing Several workers have shown that slight non progressive functional alterations result (3 4 7) According to Zollinger (9) the acute interstitial nephritis observed in cases of acute renal failure later on gives rise to chronic interstitial nephritis The follow up study that we performed on 18 patients showed that in ten of them alterations that could be characterized as chronic interstitial nephritis occurred thus confirming the opinion of Zollinger It must be taken into account that in the cases that were followed up the acute renal failure was mainly due to intoxication However two patients with appendicitis and anaphylactic shock respectively as the underlying cause developed chronic interstitial changes This we think must be considered when the prognosis of acute renal failure is discussed

## REFERENCES


1 Brun C & Munck O Lesions of the kidney in acute renal failure following shock Lancet 1 603 1957

Brun C & Olsen T S Acute renal failure In Structural basis of renal disease E Lowell Becker ed p 387 Hoeber New York 1968

3 Finkenstaedt J T & Merrill J P Renal function after recovery from acute renal failure New Engl J Med 254 10 3 1956

4 Lowe K G The late prognosis in acute tubular necrosis An interim follow up report on 14 patients Lancet 1 1086 1952

5 Oliver J Correlations of structure and function and mechanisms of recovery in acute tubular necrosis Amer J Med 15 535 1953

6 Oliver J M Dowell M & Tracy A The pathogenesis of acute renal failure associated with traumatic and toxic injury Renal ischemia nephrotoxic damage and the ischemuric episode J clin Invest 30 1305 1951

7 Price J D E & Palmer R A A functional and morphological follow up study of acute renal failure Arch intern Med 105 90 1960

8 Richet G Alagille D Nezelof C & Mery J I Physiopathologie et pathogenie des anuries hemolytiqu du post abortum J Urol med chir 62 556 1956

9 Zollinger H V Interstitial nephritis In The kidney F K Mostofi and D E Smith eds Williams & Wilkins Co Baltimore 1966

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# THE COMBINED DIURETIC ACTION OF QUINETHAZONE AND FUROSEMIDE IN CONGESTIVE HEART FAILURE

## *A Permutation Trial Test*

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*Abstract* The combined diuretic action of quinethazone and furosemide has been studied in 24 patients with congestive heart failure maintained on a constant diet. The study was performed as a permutation trial test in which the error variance is reduced while permitting the examination of drug effects separate from the variations due to different patients and varying sequence of administration of drugs The diuretic response to single drugs and to the combination of drugs in half doses showed exactly the same trend in 12 patients with slight to moderate degree of sodium retention as in 12 patients with severe sodium retention given double doses of diuretics The results obtained indicate an additive effect of the combination of diuretics in terms of osmolal clearance urinary sodium and chloride output as well as in terms of the development of hypochloraemic hypokalaemic alkalosis The diuretic action of furosemide differed significantly from that of quinethazone The renal tubular response to furosemide was characterized by (*a*) a larger increase in natriuresis with rising doses (*b*) a significant decrease of tubular reabsorption of solute free water and (*c*) a shorter duration of action Apparently the combined effect of the two drugs is most easily explained when it is assumed that the two diuretics at least in part, inhibit renal tubular sodium reabsorption at different sites in the nephron The combination of quinethazone and furosemide may prove useful in the treatment of refractory cardiac oedema when precautions are taken to avoid serious electrolyte disturbances

The present study was prompted by the clinical observation that patients with congestive heart failure resistant to usual benzothiadiazine diuretics often show a more marked natriuresis after a combination of quinethazone (2-ethyl 7-chloro-6-sulfamyl 1 2 dihydro-4-quinazolone) (4 7 16) and furosemide (4-chloro N(2 furylmethyl)5 sulfamoyl anthranilic acid) (1 2 3 13) than after larger doses of furosemide alone On this account a systematic investigation of the action of the combination of quinethazone and furosemide was found desirable

## MATERIAL AND METHODS

The subjects were adult patients with unequivocal organic heart disease of arteriosclerotic rheumatic or congenital aetiology and signs of congestive heart failure All patients had previously received diuretic treatment which was discontinued at the start of the study Digitalis medication when given previously was continued during the trial

The patients received a diet of constant composition throughout a week, containing 60 mEq sodium and chloride and 50 mEq potassium daily The fluid intake was fixed at 1500 ml per day

The urine was collected in twelve hour periods 7 a m – 7 p m and 7 p m –7 a m. and urinary excretion of water osmolal clearance free water clearance sodium potassium chloride net acid and creatinine were determined according to methods previously described (5 10) The serum osmolality sodium potassium, chloride standard bicarbonate and creatinine were determined every morning in the fasting state from the 4th to the 7th day Body weight was measured every morning

The administration of diuretics which started on the fourth day in the morning followed the scheme shown in Table I, where A indicates quinethazone (Aquamox) B furosemide (Lasix) and C the combination of quinethazone (Aquamox) and furosemide (Lasix) in half doses Through this scheme all diuretics had an equal chance to show their effects regardless of the variations caused by different patients and varying sequence of administration of drugs (8) The patients were allocated at random to one of the six sequences of drugs In order to increase statistical significance each sequence was given to two patients.

The study was divided into two parts a low dose programme (Programme I) and a high dose programme (Programme II)

Programme I included 12 patients with slight to moderate congestive heart failure After discontinuation of pre

Table I *Sequence of administration of diuretic treatments*

| No of pats | Days | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| 2 | A | B | C |
| 2 | A | C | B |
| 2 | B | A | C |
| 2 | B | C | A |
| 2 | C | A | B |
| 2 | C | B | A |

Treatments A=quinethazone B=furosemide C=quinethazone+furosemide

vious thiazide diuretics and three days of constant diet these patients showed the following control urinary excretion values per 24 hours osmolal clearance 1564 ml free water clearance −570 ml diuresis 994 ml Na 37 mEq K 46 mEq Cl 30 mEq net acid 48 mEq The diuretics were administered on the 4th–6th day of the study and the dose levels were A = quinethazone 100 mg *B = furosemide 80 mg C = quinethazone 50 mg and furo*semide 40 mg

Programme II was given to 12 patients with moderate to severe failure who had previously received more than one diuretic or furosemide in a larger dose The control 24 hour excretion values on the third day were osmolal clearance 1332 ml free water clearance −349 ml diuresis 983 ml Na 7 mEq K 61 mEq Cl 14 mEq net acid 27 mEq The dose levels of diuretics were A = quinethazone 200 mg B = furosemide 160 mg C = quinethazone 100 mg and furosemide 80 mg In order to avoid serious serum electrolyte disturbance this group was given 3 g of potassium chloride daily during the trial

In both programmes the diuretics were given in two divided doses at 8 a m and 1 p m

The statistical analysis takes advantage of the design of the experiment which allows an account for the variations due to different patients and to different sequence of administration of drugs Through analysis of variance the error variance is reduced and the significance of the effects of the treatments alone is increased. The effects of treatments may be resolved into two comparisons by means of the rules of orthogonality (8 17). The first comparison is between two groups of treatments and the second comparison is between the sum of these two treatments and the third treatment

It is a prerequisite for the design of the study that the action of the diuretics has ceased before the administration of the next treatment group Available reports appear to indicate that this holds true for quinethazone (4 9 11), as well as for furosemide (13)

## RESULTS

### A *Quantitative Effects of Diuretic Treatments*

#### 1 *Urinary output*

The mean 24 hour values for renal water and electrolyte excretion after each type of treatment

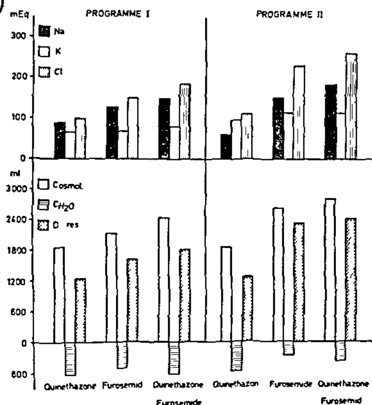



*Fig 1* Mean values for urinary electrolyte excretion osmolal and free water clearance and diuresis in relation to types of diuretic treatment

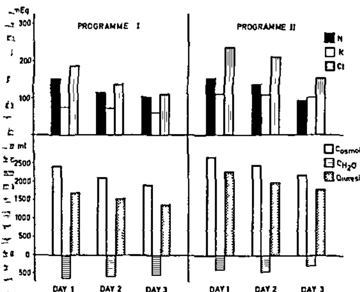



*Fig 2* Mean values for urinary electrolyte output osmolal and free water clearance and diuresis in relation to days of treatment.

are illustrated in Fig 1 and are given with statistical analysis in Table II It appears from this table that a large part of the variations in response observed is caused by different patients and by differences in days of treatment As an example the gradual reduction in mean values of response from the first to the third day of treatment are shown in Fig 2 In the following analysis the significance of the effects of treatments alone is examined after elimination of the two variables mentioned above

The mean values for osmolal clearance renal water sodium chloride and potassium outputs were higher after furosemide than after quinethazone in both programmes In programme I a significant difference was found for water and chloride outputs ($p < 0.05$) and in programme II for osmolal clearance water sodium and chloride excretion ($p < 0.01$) At the arbitrary dose levels chosen furosemide appeared to be a more potent natriochloruretic and diuretic agent than quinethazone

However after the combination of the two diuretics in half doses the mean values for osmolal clearance renal water sodium and chloride outputs were higher than after any of the single diuretics in both programmes The differences were statistically significant in comparison with quinethazone ($p < 0.01$) but did not reach the significance level in the comparison with furosemide When the effects of the combined diuretics in half doses and of the sum of the two single diuretics are compared the mean values for osmolal clearance renal water and chloride excretion are significantly higher after the combined diuretics in both programmes and the sodium excretion is significantly increased in programme II The similarity of trends in the two programmes warrants the conclusion that the combination of diuretics in half doses had a stronger natrio chloruretic and diuretic effect than the single diuretics

The excretion of potassium tended to increase with rising sodium output but statistically significant differences did not appear The net acid excretion showed no change

The negative free water clearance i e the tubular reabsorption of solute free water tended to be lower after furosemide than after other treatments In programme II in which larger doses were used the negative free water clearance was significantly lower after furosemide than after quinethazone while the value after the combined diuretics was intermediate between them (Table II and Fig 1)

## 2 *Serum osmolality and serum electrolytes*

While serum osmolality and serum sodium concentrations tended to remain unchanged during the trial a progressive tendency to hypochloraemia hypokalaemia and metabolic alkalosis was evident from the first to the third day as

Table II *Statistical analysis of renal water solute and electrolyte excretion*

| | Units | Mean 24 h values for each treatment | | | Variance ratios: Sources of variation | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | Patients | Days | Treatment |
| Significance limits | F 99 | | | | 3.29 | 5 85 | 5.85 |
| | F 95 | | | | 2.31 | 3.49 | 3 43 |
| **Programme I Urine** | | | | | | | |
| Osmolal clearance | ml/24 h | 1873 | 2130 | 2448 | 1 95 | 5 07 | 5 43 |
| Free water clearance | ml/24 h | −627 | −513 | −628 | 6 84 | 1.00 | 1 11 |
| Diuresis | ml/24 h | 1246 | 1617 | 1820 | 0 90 | 3.41 | 6.21 |
| Sodium | mEq/24 h | 88 | 127 | 147 | 0 50 | 2.30 | 3.07 |
| Potassium | mEq/24 h | 64 | 67 | 78 | 2.92 | 1 98 | 1 71 |
| Chloride | mEq/24 h | 100 | 149 | 187 | 0.53 | 5 87 * | 7.05 |
| Net acid | mEq/24 h | 37 | 58 | 46 | 0 90 | 0 13 | 1 75 |
| Creatinine | mg/24 h | 940 | 983 | 1075 | 8 11 | 3.06 | 1.49 |
| Body weight | kg | −0 16 | −0 64 | −0 93 | 0 39 | 3 35 | 5.28 |
| **Programme II. Urine** | | | | | | | |
| Osmolal clearance | ml/24 h | 1877 | 2624 | 2809 | 4 40 | 3.22 | 11 9* |
| Free water clearance | ml/24 h | −598 | −276 | −387 | 16 10 | 0.53 | 8 3 |
| Diuresis | ml/24 h | 1279 | 2345 | 24.1 | 2.09 | 2.66 | 17.07 |
| Sodium | mEq/24 h | 60 | 150 | 178 | 2.74 | 3 12 | 13.30 |
| Potassium | mEq/24 h | 94 | 111 | 111 | 4 42 | 0.40 | 2.3* |
| Chloride | mEq/24 h | 108 | 229 | 261 | 1.53 | 3.36 | 11.80 |
| Net acid | mEq/24 h | 41 | 48 | 45 | 3 43 | 0.53 | 0.69 |
| Creatinine | mg/24 h | 1004 | 1071 | 933 | 7.58 | 0 21 | 1.47 |
| Body weight | kg | −0.29 | −1 13 | −1 10 | 1 29 | 5 83 | 5 6 |

Abbreviations treatments A = quinethazone. B = furosemide. C = quinethazone + furosemide
Code for statistical significance *P* less than 0 05 *P* less than 0 01

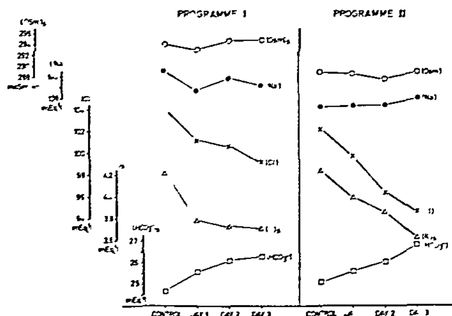



*Fig 3* Mean values for serum osmolality and electrolyte concentrations in relation to days of treatment.

| npanisons | | | |
|---|---|---|---|
| ᷈B)² | (A-C)² | (B-C)² | (A+B-2C)² |
| 0 | 8 10 | 8 10 | 8 10 |
| 5 | 4 35 | 4 35 | 4 35 |
| 6 | 10 83 | 3 31 | 8 71 |
| 5 | 0 00 | 1 68 | 0 57 |
| 6 | 12 07 | 1 50 | 7 36 |
| 9 | 5 93 | 0 67 | 3 54 |
| 0 | 3 03 | 2 00 | 3 32 |
| 9 | 14 03 | 2 72 | 9 71 |
| 7 | 0 57 | 1 21 | 0 03 |
| 9 | 2.86 | 1 33 | 2 70 |
| 0 | 10 32 | 1 41 | 6 45 |
| 4 | 21 35 | 0 88 | 10 31 |
| 19 | 6 74 | 2 03 | 0 45 |
| 76 | 27 33 | 0 12 | 10 38 |
| 2 | 24 32* | 1 34 | 12 37 |
| 4 | 3 41 | 0 00 | 1 09 |
| 70 | 21 24 | 0 25 | 10 39 |
| 18 | 0 35 | 0 33 | 0 00 |
| 18 | 0 79 | 2 95 | 2 77 |
| 37 | 7 10 | 0 18 | 1 67 |

shown in Fig 3 Apparently this pattern paralleled the degree of natriuresis and the progressive volume reduction

In accordance with this concept the alterations in serum electrolyte values were smallest after quinethazone and most marked after the combined diuretics as illustrated in Fig 4 which also gives the ranges of serum electrolyte variations

### 3 *Kidney function*

As shown in Table II the urinary creatinine excretion values per 24 hours showed no significant variation during the trial and serum creatinine levels were unchanged

### 4 *Body weight*

The weight losses during diuretic treatment followed the pattern of renal water excretion as shown in Table II In both programmes the weight loss was significantly higher after furosemide than after quinethazone In programme I the weight loss after the combined diuretics was significantly higher than after the sum of the single diuretics ($p<0.05$) Because of the decreased renal tubular reabsorption of solute free water and the subsequent high water output after furosemide in high doses a significant difference does not appear in programme II even though the sodium and chloride outputs were higher after the combined diuretics than after furosemide alone

### B *Qualitative Differences in Action of Diuretics*

As shown in Table II the double dose of furosemide used in programme II results in a higher natriuresis than the single dose used in programme I whereas the opposite result is found in the case of quinethazone This finding suggests that furosemide inhibits sodium reabsorption more extensively in the nephron than quinethazone

Analysis of the day and night portions of the

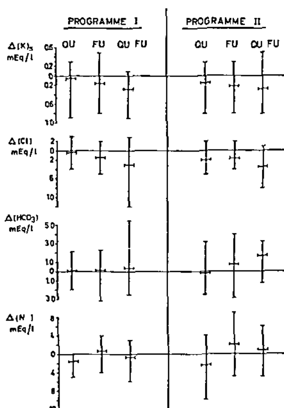



*Fig 4* Mean alues and ranges for changes in serum electrolytes in relation to types of diuretic treatments QU = quinethazone FU = furosemide QU + FU = q inethazone + furosemide

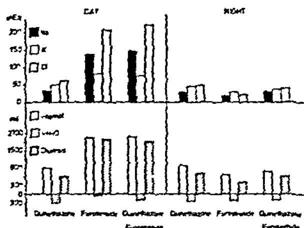


*Fig 5* Mean values for urinary electrolyte excretion, osmolal and free water clearance and diuresis in relation to types of diuretic treatment, day and night portions, programme II

urine revealed the following trends which are illustrated for programme II in Fig 5 after quinethazone administration an increase was noted both in the day and in the night portion with regard to sodium, water potassium and chloride output in accordance with the known long duration of action of this drug. After furosemide the predominant increase of sodium potassium chloride and water output took place during the day confirming the shorter duration of action of this diuretic

After the combination of diuretics in half doses the major increment of water and electrolyte excretion took place during the day but a slight increase was also noted during the night. These results were similar in both programmes and suggest an additive action of the two diuretics resulting in an increased output during the day caused by both drugs and an augmented output during the night caused mainly by quinethazone

In programme II in which larger doses of diuretics were used a striking finding was that the negative free water excretion during daytime was significantly smaller after furosemide and after the combination of diuretics than after quinethazone alone as shown in Fig 5 The decreased renal tubular reabsorption of solute free water after furosemide alone or in combination reflects the specific action of this drug on the renal concentrating mechanisms (2 5 13 14 15) and suggests that furosemide and quinethazone at least in part, act at different sites of the nephron

## DISCUSSION

The planning and the statistical treatment used in this study result in a comparative evaluation of the three diuretic regimens in which the error variance is reduced while permitting the examination of drug effects separate from the variation due to different patients and to varying sequence of administration of drugs (8)

At the arbitrarily chosen dose levels of diuretics the effect of furosemide was superior to that of quinethazone in both groups of patients in terms of osmolal clearance and of renal water sodium and chloride outputs The patterns of urinary electrolyte excretion were very similar for the two drugs which act as natriochloruretic agents and both induce an increased potassium excretion Similarly both drugs promote the same pattern of serum electrolyte changes While serum osmolality and serum sodium concentrations remain unaltered there is a tendency to hypochloraemia hypokalaemia and metabolic alkalosis These serum electrolyte changes tend to be more pronounced after furosemide reflecting the larger sodium loss induced by this diuretic

The combination of quinethazone and furosemide in half doses results in both series in mean values for osmolal clearance for sodium and chloride outputs which are higher than those of any of the single diuretics The mean values for the combination are significantly higher than those for quinethazone and these differences show higher variance ratios than the differences between the mean values for quinethazone and for furosemide alone as shown in Table II Since this trend is exactly the same in both groups of patients, it appears to indicate that the combination has a stronger natriuretic effect than either of the two single diuretics in double doses Correspondingly the serum electrolyte changes tend to be more marked after the combination than after either of the single diuretics

These findings demonstrate at least an additive effect of the combination of the two diuretics with regard to sodium chloride and total osmolal output and with respect to serum electrolyte changes, i e an algebraic summation of their effects

The possibility of a potentiating effect, i e a potentiation summation of the effects of the two drugs is present However no definite conclusion can be made since the effects of half doses of the single diuretics are not included in the trial

The fact that the natriuresis after the combination of half doses of quinethazone and furosemide is larger than after any single diuretic is most easily explained when it is assumed that the two diuretics inhibit renal tubular sodium reabsorption at least in part through different mechanisms The present study does not afford definite evidence in support of this concept but it demonstrates some important differences in the renal tubular action of the two drugs Firstly a comparison of the responses to quinethazone and furosemide in the two groups of patients shows quite clearly that the dose response curves of the two drugs are different While the double dose of quinethazone induces a smaller natriuresis in group II (with marked sodium retention) than in group I the double dose of furosemide has the opposite effect These findings are compatible with the interpretation that furosemide depresses tubular odium transport more extensively in the nephron than quinethazone (1 2 3 6 13 14 15) Secondly as shown in Table II and in Figs 1 and 5 furosemide diuresis is associated with a significant depression of renal tubular reabsorption of solute free water as compared to quinethazone diuresis This effect which tends to increase total renal water output, and may explain the thirst often mentioned by patients receiving large doses of furosemide reflects the inhibition of sodium reabsorption in the ascending limb of Henle s loop The subsequent disappearance of the hypertonicity of the renal medulla results in inability to concentrate urine and to preserve water for the body (2 13 14 15) Thirdly our study confirms that quinethazone has a natriuretic effect throughout the day and the night period as shown in Fig 5 while furosemide exerts its effect during daytime only However the combined natriuretic effect of quinethazone and furosemide is most marked during daytime and is most easily explained when it is presumed that the two drugs at least in part inhibit tubular sodium reabsorption at different sites in the nephron

Our results suggest that a combination of quinethazone and furosemide may offer advantages in the treatment of refractory cardiac oedema Similar effects of a combination of chlorthalidone and hydrochlorothiazide have been reported previously (11) However it should be taken into consideration that use of such combinations results in a more marked tendency to hypokalaemic hypochloraemic alkalosis in response to volume depletion and that these serum electrolyte disturbances may prove to be risky especially in patients receiving digitalis preparations In fact four of our patients developed bigeminy during the trial Therefore careful observation is necessary and potassium chloride supplements or addition of spironolactone or triamterene should be considered during such trials

## REFERENCES


1 Brest, A N Seller R, Ramirez, O Onesti, G & Moyer I H, Comparative diuretic eff'cacy of furosemide J New Drugs 5 3 9 1965

2 Buchborn, E & Anastasakis, S Angriffspunkt und Wirkungsmechanismus von Furosemid am distalen Nephron des Menschen. Klin Wschr 4_ 1127 1964

3 Deetjen, P Micropuncture studies on site and mod of d uretic action of furosemide Ann. N Y Acad Sci 139 408 1966

4 Frank, H, Dentler H & B rg, G Observations on the mechanism of action of a new diuretic Dru, Res. 13 56 1963

5 Goldberg, M McCardy D K, Foltz, E L & Bluemle L W Effects of ethacrynic acid (a new salu retic a ent) on renal diluting and concentrating mechanisms evidence for site of action in the loop of Henle J clin Invest 43 _01 1964

6 Heidland, A, Klutsch, K & Suzuki, F Nierenhamodynamik, Wasser und Elektrolytausscheidung nach 4-Chlor N-( furylmethyl)-5-sulfamoyl anthranit Saure Arzneimittel Forsch 14 713 1964

7 Hutcheon D E, Leonard, G B & Lomewski, A. E. B oassay of d uretics in congestive heart failure A comparison of quinethazone with hydrochlorothiazide J New Drugs 4 148 1964

8 Kal F Permutation trial of diuretics Circulation _1 717 1960

9 Kruck, F The effect of various diuretics on osmoti clearance electrolyte el mination and acid-base balance Drug Res 13 44 1963

10 Sandde E & Olesen, K Hypokalaemia, hypochloraemia and baseosis in longterm treatment of oedematous heart failure with benzothiadizine diuretics. I Incidence and pathophysiology Acta med scand 17 691 196

11 Seller R H, Fuchs, M Swartz, C, Brest, A N & Moyer J H T eatment of edema by the combined us of chemically different diuretic agents. Amer J Card ol 1_ 8 8 1963

1_ Snedecor G W Statistical methods Iowa State Medical College Ames, Iowa 19 6

13 Stason, W B Cannon P J Heinemann, H O & Laragh J H Furosemide a clinical evaluation of its diuretic action. Circulation 34 910 1966

14 Suki, W., Rector F C. & Seldin, D W.. The site of action of furosemide and other sulfonamide diuretics in the dog. J clin. Invest. 44 1458 1965
15 Suzuki, F., Klutsch, K. & Heidland, A.. Stop-flow studies on the mechanism of action of furosemide Klin. Wschr 42. 569 1964
16. Swartz, C., Seller R., Fuchs, M., B est, A. N & Moyer J H.. Five years experience with the evaluation of diuretic agents. Circulation 28 1042, 1963

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# EFFECT OF ALUMINIUM HYDROXIDE (ALUDROX) UPON SERUM CALCIUM SERUM PHOSPHORUS AND CALCIUM$^{47}$ TURNOVER IN URAEMIC PATIENTS

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*Abstract* In 11 normal persons and 16 uraemic patients serum calcium serum phosphorus and calcium pool (exchangeable calcium) were determined (Heaney and Whedon (9)) Among the 16 uraemic patients eight had inulin clearance < 0 ml/min five between 20 and 40 ml/min and three >40 ml/min Nine of the uraemic patients had serum calcium levels below normal (inulin clearance <40 ml/min) and six serum phosphorus above the normal range (inulin clearance <20 ml/min) Ten had abnormal X ray appearances of the spine and skull (halisteresis fractures) Fourteen had chronic pyelonephritis, one renal tubular acidosis and one polycystic kidneys The exchangeable calcium was 59 9 ± 13 9 mg/kg in the normals and 77 6 ± 39 4 mg/kg in the uraemic patients this difference is not significant. The patients with severe halisteresis had the highest exchangeable calcium

In four normals and 14 uraemics the effect of 6 mangesiumfree aluminium hydroxide (Aludrox) was studied, 90 ml being given daily for 1–8 months, mean period for the normals 3 5 months, for the uraemics 4 8 months During the treatment the serum phosphorus decreased there was a correlation between this decrease and the original level ($r = -0.540$) Serum calcium rose when it had originally been reduced

Exchangeable calcium increased in all the normals and in all uraemics with inulin clearance >20 ml/min. In four of the uraemic patients with inulin clearance <20 ml/min exchangeable calcium decreased In patients who showed an increase the exchangeable calcium again declined towards the initial values at the end of some months

The bone formation rate was determined in 12 of the uraemic patients (mean = 14 5 ± 8 6 mg/kg, in normals 1 ± 2.3 mg/kg this difference is not significant) Here too the highest values were found in patients with severe bone complications. In 11 of these patients the effect of aluminium hydroxide was studied (seven with inulin clearance < 0 ml/min three with inulin clearance between 0 and 40 ml/min and one with inulin clearance above 40 ml/min) The values rose in all patients with inulin clearance above 20 ml/min and in five with inulin clearance below 0 ml/min. After some time the bone formation rate declined towards the initial values

There was a correlation between exchangeable calcium and bone formation rate ( = 0 740) and between the fall in serum phosphorus and the increase in bone formation rate ($r = -0.531$)

It has previously been demonstrated by a number of authors that aluminium hydroxide may lower the elevated serum phosphorus often seen in severe renal failure (1 12) The explanation is allegedly that inorganic phosphorus is bound in the gastrointestinal tract as complex non absorbable aluminium phosphate compounds (5) Normal subjects show no or only negligible reduction of the serum phosphorus values (7)

In a previous study by the present authors (7) the administration of magnesium free aluminium hydroxide in uraemics was found to lower the serum phosphorus levels when given for six days and this effect increased with decreasing renal function If the serum calcium was reduced this level would rise At the same time there was an increase in the percental tubular re absorption of phosphorus (TRP) The calcium content of the urine also showed a tendency to rise

For the purpose of assessing the long term effect of aluminium hydroxide upon serum calcium serum phosphorus and calcium turnover estimated by Ca$^{47}$ we studied a number of uraemic subjects before and during treatment with magnesium free 6 aluminium hydroxide (Aludrox Wyeth) 90 ml daily

## MATERIAL

The material comprised 11 patients without renal osseous or endocrine disorders as well as 16 patients with renal diseases and varying degrees of uraemia. Tables I and II present the patients data

Table I *Clinical data of 11 non uraemic patients*

| No | Sex | Age | Body weight (kg) | Diagnosis | X rays of bones | Se–Ca (mEq/l) (4 5–5 5) | Se–P (mM/l) (0 9–1 5) | Se–creatinine (mg/100 ml) | Al(OH)$_3$ treatm. |
|---|---|---|---|---|---|---|---|---|---|
| 1 | ♀ | 57 | 92 | Heart disease | Not done | 4 47 | | 0 7 | – |
| 2 | ♀ | 42 | 57 | Melaena | Not done | 4 72 | 1 37 | 0 7 | – |
| 3 | ♀ | 43 | 82 | Rheumatoid arthr | No abn | 4 62 | 1 21 | 1 2 | + |
| 4 | ♀ | 54 | 66 | Prolapse of the disc | Not done | 5 09 | 1 21 | 1 0 | + |
| 5 | ♀ | 25 | 62 | Duodenal ulcer | Not done | 4 90 | | 1 1 | – |
| 6 | ♂ | 51 | 112 | Art hypertension | No abn | 5 22 | 1 16 | 1 1 | + |
| 7 | ♀ | 34 | 51 | Duodenal ulcer | No abn | 4 91 | 0 80 | 0 7 | + |
| 8 | ♀ | 55 | 49 | Myositis | No abn | 4 68 | | 0 9 | – |
| 9 | ♂ | 67 | 51 | Diabetes mell | Not done | 4 63 | 1 13 | 1 0 | – |
| 10 | ♂ | 48 | 56 | Gastritis | No abn | 4 90 | 1 19 | 0 9 | – |
| 11 | ♀ | 70 | 56 | Heart disease | No abn | 4 81 | 1 24 | 0 9 | – |

The renal function was assessed by inulin clearance (4) Eight of the patients had a clearance of < 20 ml/min five between 20 and 40 ml min and three > 40 ml/min All the uraemic patients had pyelonephritis except for two who were suffering from polycystic kidneys and tubular acidosis respectively (Table II cases 1 and 13) The age range was 25–74 years and the age distribution in the two groups is comparable (average 49 6 and 57 1 years respectively) Nine of the patients with renal failure had reduced serum calcium six elevated and one reduced serum phosphorus Ten of the patients with renal disease had abnormal X ray appearances of the spine and skull (halisteresis fractures) Only four of the control patients were treated with Aludrox, while 14 of the patients with renal failure were investigated two to four times during this treatment. The average treatment period was 3 5 months (2–6 months) for the control group and 4 8 months (1–8 months) for the uraemic group During the treatment the patients were on the ordinary hospital diet and were kept in bed

## METHODS

Before and repeatedly during the Aludrox treatment the following investigations were performed serum calcium by the EDTA murexide method (19) serum phosphorus by the method of Muller (16) and calcium turnover determination using Ca by the technique of Heaney and Whedon (9)

30–40 μC Ca Cl having a specific activity of 90 mC/g was administered intravenously Venous blood was drawn three and five hours after the injection and daily during the subsequent ten days for determination of radioactivity serum calcium and serum phosphorus. The radioactivity was determined in a well type scin-

Table II *Clinical data of 16 uraemic patients*

| No | Sex | Age | Body weight (kg) | Diagnosis | X rays of bones | Se–Ca (mEq l) (4 5–5 5) | Se–P (mM l) (0 9–1 5) | Se–creat (mg/100 ml) (< 1 5) | Alk phosph (< 3 2U) | Inulin clear | Al(OH) treatm. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | 54 | 46 | Bilat polycystic kidneys | Halisteresis (severe) | 4 30 | 2 34 | 15 6 | 2 0 | 4 | + |
| 2 | ♀ | 63 | 60 | Chronic pyelonephritis | No abn | 4 45 | 1 81 | 6 8 | 1 9 | 5 | + |
| 3 | ♂ | 69 | 71 | Chronic pyelonephritis | Halisteresis | 3 83 | 1 63 | 4 1 | 3 6 | 11 | – |
| 4 | ♀ | 64 | 51 | Chronic pyelonephritis | Halisteresis | 4 38 | 1 97 | 4 2 | 3 3 | 14 | – |
| 5 | ♀ | 64 | 56 | Chronic pyelonephritis | No abn | 4 48 | 1 32 | 3 9 | 1 9 | 15 | – |
| 6 | ♀ | 56 | 69 | Chronic pyelonephritis | Halisteresis | 4 15 | 1 59 | 5 5 | 2 8 | 16 | + |
| 7 | ♀ | 65 | 68 | Chronic pyelonephritis | No abn | 4 88 | 1 04 | 3 4 | 2 7 | 17 | – |
| 8 | ♀ | 73 | 40 | Chronic pyelonephritis | Spont fract | 3 19 | 1 78 | 4 5 | 7 1 | 19 | + |
| 9 | ♀ | 61 | 42 | Chronic pyelonephritis | Cysts of the iliac bone | 4 55 | 1 0 | 2 1 | 3 9 | 22 | + |
| 10 | ♂ | 45 | 50 | Chronic pyelonephritis | Halisteresis | 4 45 | 0 90 | 2 3 | 1 3 | 24 | + |
| 11 | ♀ | 45 | 47 | Chronic pyelonephritis | Spont fract | 4 77 | 1 35 | 2 0 | 2 5 | 31 | + |
| 12 | ♀ | 74 | 68 | Chronic pyelonephritis | Halisteresis (mild) | 4 56 | 1 11 | 2 [illegible] | 2.2 | 3[illegible] | – |
| 13 | ♀ | 40 | 48 | Renal tub acidosis | Halisteresis (severe) | 3 95 | 0 56 | 2 2 | 4 4 | 39 | + |
| 14 | ♀ | 43 | 52 | Chronic pyelonephritis | No abn | 4 72 | 1 05 | 1 4 | 1 1 | 42 | – |
| 15 | ♀ | 45 | 66 | Chronic pyelonephritis | No abn | 5 00 | 1 0[illegible] | 1 5 | 2 1 | 50 | + |
| 16 | ♂ | 52 | 42 | Chronic pyelonephritis | No abn | 4 60 | 1 11 | 1 7 | 1 5 | 60 | + |

tillation crystal detector connected to a single channel gamma spectrometer thus eliminating radiation from the daugther decay product scandium Urine and stools were collected daily and the radioactivity in the urine was determined in a volume of 1 1 in the serum 5 ml The results are stated in per cent of the administered dose standards of the same volume being counted at the same time The studied volume of stool was 100 or 00 ml after mixing with water depending upon the quantity of the stool

On the basi of the findings the following parameters were calculated. On a semilogarithmic system we plotted the days after the injection as the abscissa and the spec f c activity (radioactivity in per cent of the dose per 1 s rum mEq Ca/1 serum) as th ordinate From 48 hours after the injection the curve is rectilinear but usually it deflects 5–6 days after the injection and there after runs a flatter course (9 10) Th s phenomenon has not been entirely e ucidated (Fig 1) The change in the slope of the curve must be due to a return to the blood of calc um having a higher specific activity Mineral of h gh specific activity is found in new formed bony tissue However bone resorption is a slow process which cannot be responsible for the change in the slope of the curve a early as five days after the injection The explanation must be that calcium of high specific activity from the bony tissue is exchanged with the isotope from the blood As a matter of fact labelled calcium settles in a high concentration on the bone absorption surface and appears to be an exchangeable fraction (10) The calcium

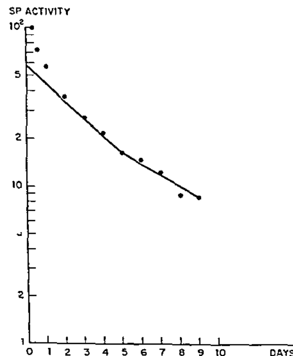



*Fig 1* Specific activity of serum calcium in relation to time from injection of Ca *Abscissa d ys Ordinate* specific activity in a logarithmic scale

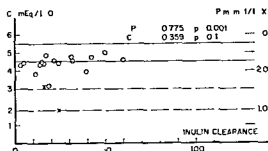



*Fig 2* Relationship between inulin c earance serum calcium, and serum pho phorus Abscissa inulin clear anc in ml/min Ordinate left serum calcium mEq/l right, serum phosphorus mM/l

pool (exchangeable calcium) was calculated on the basis of the ordinate intercept of the first rectilinear segment of the curve Calcium (mEq) pool = administered dose/ specif c activity in serum at zero time Multiplication by 20 gives the pool in mg (*E*) It seems to comprise partly calcium in the extracellular fluid partly some calc um in the soft tissues and partly certa n small amounts of calcium in the bony tissue chiefly on the absorption sur faces (9 17)

On the basis of the pool we calculated the quantity of calcium in mg which leaves the calcium pool in the course of 24 hours $(Ek)$ = calcium turnover = calcium pool in mg × $k$ where

$$k = \frac{\ln 2}{T/2}$$

On the basis of the calcium turnover the bone forma tion rate may be calculated BFR = $Ek$ $(1 - fu - fs)$ where $fu = Fu\ (t - t)/e^{kt}\ \ e^{kt}$ and $fs = Fs\ (t - t_1)\ e^{kt} - e^{kt}$ $Fu(t - t)$ and $Fs(t - t_1)$ = the percental quantity of the radioactivity excreted in the urine and faeces from 48 hours after the injections $(t)$ until the curve deflects $(t)$ $e = 2\,718$ and $kt$ and $kt$ =

$$\frac{\ln 2}{T_1/2} \text{ and } \frac{\ln 2}{T_1/2}$$

where $T$ 2 and $T/2$ are the half lives in days for the two segments of the curve (3)

Moreo er the patients had repeated determinations of serum creatinine alkaline phosphatase electrophoresis, and X ray examinations of the hand skull and spine to assess the bony structure

## RESULTS

Fig 2 gives the relationship between serum cal cium serum phosphoru and inulin clearance in uraemics before the treatment All nine uraemics with a reduced serum calcium level had an inulin clearance below 40 ml/min The serum albumin level was normal in all but one (case 9 Table II) in whom it was 2 5 g 100 ml while the serum calcium concentration in this case was normal

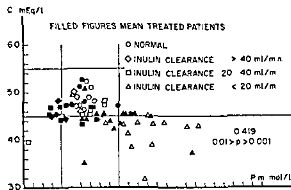



*Fig 3* Relationship between serum calcium and serum phosphorus Abscissa serum phosphorus m*M*/l Ordinate serum calcium mEq/l

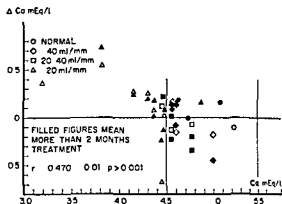



*Fig 5* Relationship between serum calcium and increase in serum calcium Abscissa serum calcium in mEq l Ordinate change in serum calcium during aluminium hydroxide treatment in mEq/l

There was a tendency to decreasing serum calcium concentrations with decreasing inulin clearance However this relationship was not significant ($r = +0\ 359$ $p > 0\ 1$) As far as serum phosphorus was concerned the six uraemics with elevated levels had an inulin clearance below 20 ml/min A correlation was found between inulin clearance and serum phosphorus level ($r = -0\ 775$ $p < 0\ 001$)

The relation between serum phosphorus and m calcium in all patients untreated as well as treated with aluminium hydroxide is apparent from Fig 3 There was a negative correlation $r = -0\ 419$ $0\ 01 > p > 0\ 001$ When considering the untreated patients separately there was also a negative correlation $r = -0\ 446$ $p < 0\ 001$ just as for all the uraemics treated and untreated ($r = -0\ 417$ $0\ 01 > p > 0\ 001$) When considering the untreated uraemics alone however the correlation was not significant ($r = -0\ 392$ $0\ 1 > p > 0\ 05$)

The relation between serum phosphorus and alterations in this concentration during aluminium hydroxide treatment is apparent from Fig 4 This shows a correlation ($r = -0\ 540$ $0\ 01 > p > 0\ 001$) the decrease being the greater the higher the serum phosphorus level prior to the institution of treatment In 11 of the uraemic patients the investigations were performed during treatment both when it had been administered for less than two months and when it had been administered longer In nine the decrease in serum phosphorus was more marked during the shorter treatment period In other words the effect appeared to

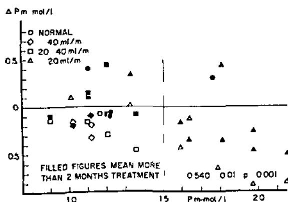



*Fig 4* Relationship between serum phosphorus and a fall in serum phosphorus Abscissa serum phosphorus m*M*/l Ordinate change in serum phosphorus during aluminium hydroxide treatment in m*M*/l

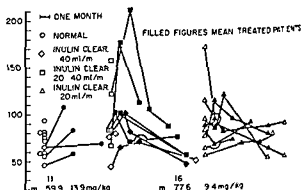



*Fig 6* Exchangeable calcium before and during treatment with aluminium hydroxide Diagram on the left normals Diagram in the centre inulin clearance > 20 ml/min Diagram on the right inulin clearance < 20 ml/min Ordinate exchangeable calcium in mg/kg

wear off The controls showed a slight fall in the serum phosphorus concentration

Fig. 5 sets out the relation between serum calcium and alterations in this concentration during the treatment In this respect, too there was a correlation ($r = -0.470$ $0.01 > p > 0.001$) though less marked than for serum phosphorus The increase in serum calcium was most pronounced when the level was lowest. Here the tendency to a greater effect during short treatment periods was not apparent

Fig 6 presents the calcium pool = exchangeable calcium ($E$) in the 11 controls and 16 uraemics. $m$ was $59.9 \pm 13.9$ mg/kg and $77.6 \pm 39.4$ mg/kg respectively i e no significant difference ($t = 1.42$ $p > 0.1$) No correlation was demonstrated between the size of the pool and that of the inulin clearance ($r = 0.1403$ $p > 0.1$) Patients having very large pools (> 100 mg/kg) had pronounced skeletal changes. This refers to cases 1 and 13 (marked halisteresis) case 9 (cysts of the iliac bone) and case 8 (spontaneous fractures) Cases 8 9 and 13 had the highest alkaline phosphatase values in the entire material (Table II)

During the administration of aluminium hydroxide to four of the control patients all showed a considerable increase in pool size On the other hand, the findings varied in the uraemics Nine showed an increase and four a decrease. It was striking that the four in whom the pool size decreased had inulin clearance below 20 ml/min In cases where the pool increased, there was later a decrease towards the initial values.

Fig. 7 illustrates the calcium turnover = the number of mg calcium which leave the pool in the course of the 24 hours ($Ek$) in order to be absorbed by the bones or to be excreted in the urine or stool $m$ was $13.4 \pm 3.1$ mg/kg and $15.1 \pm 7.5$ mg/kg respectively in the two groups, i.e. not significant differences ($t = 0.72$ $p > 0.1$) Three of the uraemic patients had greatly elevated values (> 20 mg/kg in the 24 hours) (cases 8 9 and 13) These patients had greatly elevated alkaline phosphatases too During the aluminium hydroxide treatment they showed changes similar to those in the calcium pool The levels increased in the four control patients and in ten of the uraemics, while they fell in four Values which showed an initial increase fell later just as in the case of the pool

The bone formation rate was determined in six

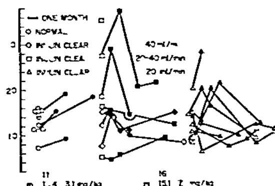



*Fig 7* Calcium turnover before and during treatment with aluminium hydroxide Diagram on the left. normals. Diagram in the centre inulin clearance > 20 ml min. Diagram on the right. inulin clearance < 20 ml min. Ordinate calcium turnover in mg kg 24 h.

of the controls and 12 of the uraemics. In the remaining patients the $Ca^{4}$ excretion in the urine or stool was not studied In the other control patients the $Ca^{4}$ excretion in the urine ranged from 9.6 to 28.1 % of the dose $m = 14.5$ % and that in the stool from 4.7 to 15.3 % $m = 10.4$ % In the uraemics the urinary excretion ranged from 0.30 to 4.6 % $m = 1.5$ % and the faecal excretion from 0.4 to 17.0 % $m = 6.0$ % The mean values for BFR in the two groups of patients were $12.2 \pm 2.3$ mg/kg 24 hours and $14.5 \pm 8.6$ mg kg/24 hours (Fig. 8) a difference which is not significant ($t = 0.64$ $p > 0.1$) There was no correlation between the inulin clearance and the BFR ($r = +0.1431$ $p > 0.1$) Four uraemics had greatly elevated levels (exceeding 17 mg/kg) (cases 8 9 11 and 13) Three of them were iden-

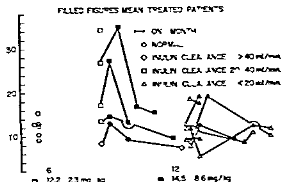



*Fig 8* Bone formation rate before and during aluminium hydroxide treatment. Diagram on the left. normals. Diagram in the centre inulin clearance > 20 ml min. Diagram on the right inulin clearance < 20 ml min. Ordinate bone formation rate in mg kg/24 h.

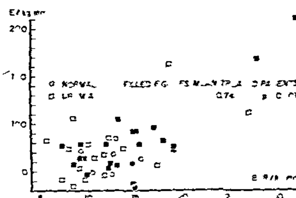


*Fig 9* Relationship between exchangeable calcium and bone formation rate Abscissa bone formation rate in mg kg 24 h. Ordinate exchangeable calcium in mg kg.

tical with the patients having a greatly increased *pool and calcium turnover The fourth (case 11)* had spontaneous fractures During the aluminium hydroxide treatment the values increased in nine out of 11 patients, while they fell in two (patients with severe uraemia)

An investigation was also made as to whether a correlation was demonstrable between the size of the calcium pool (*E*) and the quantity leaving the pool in the 24 hours. This investigation showed a positive correlation ($r = +0.631$ $p < 0.001$) Moreover we analysed the correlation between the pool and bone formation rate (BFR) (Fig 9) and between *Ek* and BFR. Here too there was a positive correlation ($r = -0.740$ $p < 0.001$ and $r = -0.971$ $p < 0.001$)

No correlation was found between alterations *in serum phosphorus and calcium pool (E) during* the aluminium hydroxide treatment ($r = -0.004$ $p > 0.1$) However uraemics with an inulin clearance exceeding 20 ml/min and the control patients showed a negative correlation ($r = -0.6$.. $0.02 > p > 0.01$) In the entire patient material there was a negative correlation between the changes *in serum phosphorus and in the calcium* turnover (*Ek*) and bone formation rate (BFR) respectively ($r = -0.373$ $0.05 > p > 0.02$ and $r = -0.531$ $0.05 > p > 0.02$) (Fig 10) Between changes in serum calcium during the treatment and changes in the calcium pool calcium turnover and bone formation rate no correlation was demonstrable ($r = -0.294$ $0.1 > p > 0.05$ $r = -0.115$ $p > 0.1$ and $r = -0.268$ $p > 0.1$).

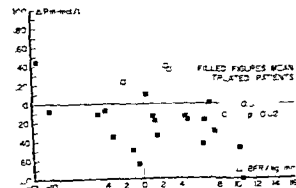


*Fig 10* Relationship between fall in serum phosphorus and increase in bone formation rate. Abscissa bone formation rate in mg kg 24 h. Ordinate change in serum phosphorus in mM L

## CONCLUSION AND DISCUSSION

Our investigations indicate that aluminium hydroxide lowers the serum phosphorus concentration—the more the higher it is. At the same time the serum calcium concentration increases if it has been reduced

The $Ca^{47}$ studies indicate that the calcium pool = exchangeable calcium (*E*) the quantity of *calcium which leaves the pool in the 24 hours (Ek)* and the bone formation rate (BFR) do not differ significantly in the control and the uraemic patients, although patients with severe osseous changes do show high values. There was no relation to the degree of uraemia. During aluminium hydroxide treatment there was an increase in the parameters, though inconstant in the severe uraemics. A correlation was found between the decrease in serum phosphorus and the increase in *E Ek* and *BFR.*

The decrease in serum phosphorus is presumably due to binding of the phosphorus in the gastrointestinal tract as heavily soluble complex aluminium phosphate compounds (5) Accordingly the phosphorus binds less calcium in the gastrointestinal tract, so that the formation of insoluble $Ca_3(PO_4)_2$ is reduced. Thus, there is possibly more calcium available for absorption *and this perhaps explains why the serum calcium* level increases when it has already been reduced (12, 13 14 15 18)

Our normal values for the calcium pool are somewhat lower than those found by Bauer et al (2) and by Heaney and Whedon (9) but their material was but small The BFR values from in the present study are somewhat higher Compared with Bernzel et al (3) there is agreement

with our calcium pool values which are higher than those reported by Haymovitz et al (8) There is also agreement in respect of the BFR values with Bentzel et al (3) As already mentioned we found in the uraemics that the pool *Ek* and BFR did not differ with certainty from those in the control patients although patients with radiological skeletal changes did have high values There is agreement with Kaye and Silverman s $Ca^{47}$ studies (11) on six control patients and 11 uraemics although their calcium pool in the control patients was somewhat higher and the BFR values some what lower than ours These authors also found the faecal excretion to be the same in controls and uraemics during the first four days of the studies (7 2 and 8 1 % of the administered dose) while th urinary excretion was far higher in the control patients (11 5 and 1 5 ) Our findings show quite good conformity with theirs the mean values for urinary excretion being 14 6 and 1 5 % of the dose administered to controls and uraemics The fact that uraemics with a high pool and BFR showed pronounced osseous changes also accords with the experience of Stanbury and Lumb (18)

The increase found in the present study in cal cium pool *Ek* and BFR in control patients and some of the uraemics on aluminium hydroxide treatment may possibly be explained as already mentioned by an increased supply of soluble cal cium in the gastrointestinal tract so that more is absorbed The explanation why some of the patients with severe uraemia did not exhibit this tendency is perhaps that in such patients the ab sorption of calcium from th intestine is reduced (11 13 18) A contributory factor in the increase may be the fall in serum phosphorus which makes the calcium phosphorus product fall and this may entail an increased loss of calcium from the bones to the extracellular fluid In this respect Lotz et al (14) demonstrated that long term treatment of normal with aluminium hydroxide may cause de calcification of the bones That the changes in the calcium pool *Ek* and BFR during aluminium hydroxide treatment are elicited by way of changes in serum phosphorus is indicated by the demon strated correlation b tween changes in serum phosphorus and changes in *Ek* and BFR On the other hand there was no correlation between changes in serum calcium and alterations in the pool *Ek* and BFR In this connection it may be mentioned that Kaye and Silverman (14) could not find any correlation between serum calcium and the calcium pool in uraemic subjects

The reason why the parameters later decrease towards the initial values is possibly that the ef fect of aluminium hydroxide gradually wears off assessed by the fall in serum phosphorus Possibly compensatory mechanisms such as reduction in parathyroid function may be contributory

The correlation found between the calcium pool and the BFR is in keeping with the findings of Heaney and Whedon although the correlation is somewhat less marked in our study We tried to ascertain changes if any in bony structure during the long term aluminium hydroxide treatment Three uraemic patients (cases 3 4 and 9) showed evidence of such changes halisteresis increa ing during the treatment A total of nine uraemics (cases 3 4 6 8 9 10 11 12 and 15) showed an increase in alkaline phosphatases during the treatment possibly as a sign of increased osteo blastic activity as a link in increasing osteomalacic changes In this connection it may be mentioned that in guinea pigs Cox et al (5) demonstrated that osteomalacia may result from such treat ment. According to Dent et al (6) rickets may develop from phosphate deficiency and lastly Lotz et al (14) have reported as already stated that long term administration of aluminium hy droxide to normal subjects may give rise to osteo malacia as one link in phosphate depletion Dur ing long-contiued u e of this treatment such pos sible effect upon the skeletal system must of course be borne in mind and the treatment can therefore hardly be recommended for uraemics who show pre-existing X ray signs of major skele tal changes

## ACKNOWLEDGEMENT

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## REFERENCES

1 Allwall N Acta chir scand 108 95 1954
Bauer G C H., Carl son A & Lundquist, B Acta med. scand. 158 143 1957
3 B ntzel C J Carbon P P & Rosenberg, L. J clin. Invest. 43 _13_, 1964
4 Bøjes n, E. A method for determinat on of inul n in plasma and urine Acta med scand. S ppl _66 75 195_

5 Cox G J Dodds M L Wigman H B & Murphy F J J biol Chem 92 11 1931
6 Dent C E Harper C M & Philpot, G R Quart. J Med 30 1 1966
7 Friis T Hahnemann S & Weeke E Acta med scand 183 497 1968
8 Haymovitz A & Horwith M J clin Endocr 24 4 1964
9 Heaney R P & Whedon G D J clin Endocr 18 1248 1958
10 Jowsey J J clin Endocr 25 1408 1965
11 Kaye M & Silverman M J lab clin Med 66 535 1965
12 Lindholm T Acta med scand 172 75 1962
13 Liu S H & Chu H J Medicine (Baltimore) 22 103 1943
14 Lotz, M Zisman E & Bartter F C New Engl J Med 278 409 1968
15 Merrild J P J chron Dis 5 138 1957
16 Muller E Hoppe Seylers Z physiol Chem 237 35 1935
17 Shorr E & Carter A C JAMA 144 1549 1950
18 Stanbury S W & Lumb G A Medicine (Baltimore) 41 1 1962
19 Wilkinson R H J clin Path 10 126 1957

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# BLOOD FLOW IN SUBCUTANEOUS FAT TISSUE IN PATIENTS WITH DIABETES MELLITUS

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*Abstract* A comparison of the abdominal subcutaneous fat tissue blood flow (FBF) has been made in 13 patients with diabetes mellitus and in 29 healthy individuals As the metabolic state may influence the tissue blood flow as well as vice versa, a possible relationship between FBF and the arterial levels of glucose lactate pyruvate $\beta$ hydroxybutyrate free fatty acids and aceto-acetate was searched for

The blood flow was calculated from the elimination curves from locally injected radioactive xenon.

The average blood flow was significantly ($p<0.001$) higher in the diabetics than in the healthy individuals There was a significant correlation between FBF and the abdominal skin temperature both in diabetics and in healthy individuals. The skin temperature was significantly higher in the diabetics.

The administration of insulin to the diabetics lowered the FBF Prolonged fasting increased FBF in the healthy controls as judged from observations after 17 hours of fasting Fasting for 34 hours did not further change the FBF No correlation was observed between the FBF and the arterial concentration of any of the substrates analyzed

It is well known that changes in the metabolic situation influence the blood flow in different tissues as for example that the blood flow in skeletal muscle increases during hypoglycemia

The question whether the metabolic disturbances per se in diabetes mellitus influence the blood flow in different tissues remains to be answered

Observations have previously been reported that there are marked differences in the abdominal subcutaneous fat tissue blood flow between diabetics and normal individuals (preliminary results were reported at the Sixth International Diabetes Congress in Stockholm 1967 (Excerpta Medica International Congress Series no 140 1967)) Furthermore the present data suggest that fat tissue blood flow increases during prolonged starvation as judged from studies on healthy individuals

## MATERIAL AND METHODS

The material comprises 13 diabetics eight males and five females aged 17–38 years, and 29 healthy individuals, 27 males and two females, aged 19–37 years Table I shows the characteristics of the diabetic patients The diabetics were treated with a conventional diet low in sugar and fat, rich in vegetables and protein The healthy individuals had at repeated clinical examinations shown no signs of any disease

In all individuals the abdominal skinfold thickness arterial blood pressure heart rate body weight and abdominal subcutaneous fat tissue blood flow (FBF) were determined. Abdominal skin temperature was measured in all diabetic subjects and in 12 healthy controls The investigations were performed in the morning when the subjects had been fasting 10 hours i e since a slight meal (two sandwiches and one glass of milk) at 10 p m. the evening before the investigation. The diabetic subjects had not taken any insulin or oral antidiabetic drugs for the last 24 hours. All individuals were studied at rest

In order to investigate possible relationships between FBF and the metabolic state of the individual simultaneously with the FBF determinations, analyses of the arterial concentrations of some substrates of importance especially in the metabolism of carbohydrates and fat were performed in 12 of the diabetics (7 males and 5 females 18–38 years old) and in 14 of the healthy individuals (12 males and 2 females 19–37 years old) In 12 of these 14 healthy individuals these measurements were repeated at 3 p m and at 8 a m. the next morning i e after 17 and 34 hours of fasting respectively In six of the diabetics the measurements were repeated during 30 min after i v injection of 0.03 IU per kg body weight of crystalline insulin, crystallized twice and claimed to be free of glucagon (Vitrum)

The blood flow was determined by local injection of radioactive xenon the elimination rate of which was followed by recording the $\gamma$-activity The method is based on the technique of Kety (9) modified by Lassen (10)

Table I *Characteristics of the diabetic patients*

| Diabetic subjects | E S | L A | A M | E R | N O | R W | S M | B O | G R | M J | A L | C S | B H |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | 17 | 22 | 17 | 32 | 22 | 38 | 23 | 27 | 26 | 28 | ?5 | 27 | 36 |
| Sex | ♂ | ♂ | ○ | ○ | ○ | ♂ | ♀ | ♂ | ♂ | ♀ | ♀ | ♀ | ♀ |
| Abdominal skinfold thickness mm | 5 7 | 13 0 | 8 0 | 10 7 | 7 2 | 7 8 | 7 3 | 6 3 | 7 9 | 10 9 | 18 1 | 17 4 | 31 6 |
| Obvious signs of angiopathy | – | – | – | – | + | + | – | + | – | + | + | + | – |
| Duration of diabetes y | 0 7 | 22 | 12 | 0 3 | 8 | 10 | 0 1 | 10 | 13 | 17 | 17 | 22 | 5 |
| Daily doses of insulin IU | – | 76 | 48 | – | 60 | 36 | 48 | 64 | 42 | 36 | 28 | 48 | – |
| Peroral antidiabetic drugs | + | – | – | – | – | – | – | – | – | – | – | – | + |

and Andrée Larsen et al. (1) Our modification of the method was earlier described and discussed (8)

*0 05 ml Xe*[133] *(approximately 50 μCi) in saline* was injected with a fine needle into the middle of the sub cutaneous layer in the lower part of the abdomen The γ-activity from the isotope depot was registered with a scintillation crystal detector The elimination curves from fat tissue were strictly monoexponential under the ex perimental conditions used as we were able to show in several ways (8)

The abdominal skinfold thickness was measured by a Harperden s caliper (5) abdominal skin temperature by the use of a thermoelectric instrument, blood glucos by a glucose oxidase method, lactate (6) pyruvate (11) and β hydroxybutyrate ( ) enzymatically free fatty acids (4) and aceto-acetate (13) colorimetrically Plasma total cholesterol total phospholipids and triglycerides were determined as earlier described (1?) In the studies when insulin was given the blood sampling was performed 15 min after the insulin administration i e in the middle of the period during which FBF was measured

## RESULTS

When measured in the morning after 10 hours of fasting the abdominal subcutaneous fat tissue blood flow (FBF) was in the diabetics 14 9 ± 2 92 ml/(100 g × min) significantly ($p < 0\ 001$) highe than in the healthy individuals 6 4 ± 0 58 ml (100 × min)

The administration of insulin to six of th diabetics lowered the FBF significantly ($p < 0\ 05$ (Table II)

Prolonged fasting for 17 hours increased th FBF significantly ($p < 0\ 01$) in the healthy con trols Further prolongation of the fasting did not however further increase the FBF as judged from the measurements after 34 hours of fastin (Table III)

The abdominal skin temperature was significant ly ($p < 0\ 05$) higher in the diabetics (33 8 ± 0 26 (Mean ± s E)) than in the healthy controls (32 8 ± 0 26 )

Fig 1 illustrates the relationship between FB and abdominal skin temperature after 10 hours o fasting in both diabetics and healthy individual

Table II *Fat tissue blood flow in six of the diabetic patients before and after insulin administration*

| Diabetic subjects | FBF (ml/(100 g min)) Before insulin admin | After insulin admin |
|---|---|---|
| 1 | 19 . | 6 9 |
| 2 | 21 0 | 16 5 |
| 3 | 11 5 | 7 9 |
| 4 | 7 5 | 1 9 |
| 5 | 9 8 | 1 0 |
| 6 | 2 5 | 2 0 |
| | $p < 0\ 05$ | |

Table III *Fat tissue blood flow in 12 of the health individuals after 10 17 and 34 hours of fasting*

| Healthy individuals | FBF (ml/(100 g min)) Fasting 10 h | 17 h | 34 h |
|---|---|---|---|
| 1 | 6 7 | 12 4 | 6 9 |
| 2 | 2 7 | 17 7 | 7 8 |
| 3 | 11 1 | 25 6 | 34 3 |
| 4 | 5 0 | 11 5 | 5 7 |
| 5 | 8 1 | 12.4 | 8 5 |
| 6 | 6 4 | 14 1 | .1 6 |
| 7 | 5 4 | 7 0 | 13 1 |
| 8 | 7 1 | 8 5 | 12.8 |
| 9 | 4 6 | 7 7 | 5 5 |
| 10 | 3 1 | 6 . | 6 2 |
| 11 | 11 9 | 1 8 | 17 3 |
| 12 | 8 3 | 11 9 | 11 7 |
| | $p < 0\ 01$ | *NS* | |

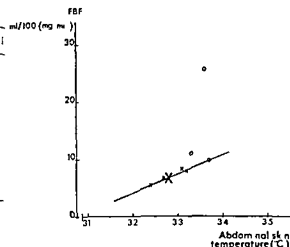



*Fig 1* Relation between fat tissue blood flow and abdominal skin temperature in diabetics (O) and healthy individuals (x) Regression line where the large X indicates the point (x ȳ) is shown for the healthy individuals.

There was a significant ($p < 0.01$) correlation between FBF and skin temperature in the healthy individuals There was also a correlation though less significant ($p < 0.05$) between these two parameters in the diabetics

There was no significant difference in abdominal skinfold thickness between the diabetics (12 4±2 44 mm (Mean±S E)) and the healthy individuals (12 2±1 29 mm) There was a significant ($p < 0.001$) negative correlation between FBF and skinfold thickness in the healthy individuals (Fig 2) This negative correlation existed also in the diabetics although with less significance ($p < 0.05$)

*Arterial Substrate Levels*

*In the diabetics* the fasting glucose level was found to be 251±25 1 mg per 100 ml of blood (Mean±S E $n = 12$) lactate 0 76±0 088 m$M$ in blood ($n = 4$) pyruvate 0 05±0 012 m$M$ in blood ($n = 4$) free fatty acids 0 62±0 061 m$M$ in plasma ($n = 12$) aceto-acetate 6 0±2 14 mg per 100 ml blood ($n = 6$) total plasma cholesterol 227±17 8 mg per 100 ml plasma ($n = 8$) total phospholipids 235±18 3 mg per 100 ml plasma ($n = 8$) and triglycerides 121±26 8 mg per 100 ml plasma ($n = 8$)

The insulin administration to six of the diabetics decreased the glucose level slightly from 278±45 1 to 263±44 8 mg per 100 ml blood and the free fatty acid level decreased from 0 70±0 069 to 0 53±0 103 m$M$ The level of acetoacetate and plasma triglycerides did not change within the 15 min period studied

*In the healthy controls* the glucose level was 88±6 5 mg per 100 ml blood ($n = 12$) in the morning after 10 hours of fasting lactate 0 97±0 098 m$M$ in blood ($n = 5$) pyruvate 0 07±0 0068 m$M$ in blood ($n = 5$) free fatty acids 0 41±0 044 m$M$ in plasma ($n = 12$) aceto-acetate 0 72±0 046 mg per 100 ml blood ($n = 12$) $\beta$ hydroxybutyrate 0 62±0 078 mg per 100 ml blood ($n = 3$) total

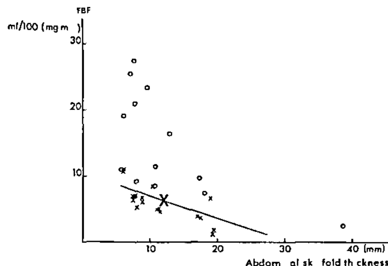



*Fig 2* Relation between fat tissue blood flow and abdominal skinfold thickness in diabetics (O) and healthy individuals (x) Regression line where the large X indicates the point (x y) is shown for the healthy individuals.

Table IV *Arterial substrate concentration in 12 of the healthy individuals after 10 17 and 34 hours of*

| Healthy individuals | Glucose (mg/100 ml blood) Fasting 10 h | 17 h | 34 h | Lactate (mM in blood) 10 h | 34 h | Pyruvate (mM in blood) 10 h | 34 h | Free fatty acids (mM in plasma) 10 h | 17 h | 34 h | Aceto-acetate (mg/100 ml blood) 10 h | 17 h | 34 h | β-hydroxy-butyrate (mg 100 ml blood) 10 h | 34 h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 94 | — | 96 | 0 73 | 0 85 | 0 04 | 0 06 | 0 58 | — | 0 82 | 1 17 | — | 3 79 | — | — |
| 2 | 98 | — | 98 | 0 90 | 0 83 | 0 08 | 0 06 | 0 45 | — | 0 49 | 0 65 | — | 1 93 | 0 5? | 2 0( |
| 3 | 96 | — | 76 | 1 02 | 0 83 | 0 07 | 0 06 | 0 31 | — | 1 47 | 0 66 | — | 8 02 | 0 77 | 17 0 |
| 4 | 91 | — | 83 | 0 90 | 0 47 | 0 07 | 0 03 | 0 37 | — | 1 04 | 0 66 | — | 4 15 | 0 57 | 6 ( |
| 5 | 82 | — | 71 | 1 32 | 0 69 | 0 08 | 0 05 | 0 33 | — | 0 50 | 0 69 | — | 5 52 | — | — |
| 6 | 80 | 78 | 75 | — | — | — | — | 0 38 | 0 41 | 1 09 | 0 70 | 1 32 | 3 09 | — | — |
| 7 | 82 | 78 | 64 | — | — | — | — | 0 19 | 0 37 | 1 27 | 0 60 | 1 13 | 10 97 | — | — |
| 8 | 93 | 80 | 76 | — | — | — | — | 0 36 | 1 15 | 0 79 | 0 68 | 1 88 | 6 46 | — | — |
| 9 | 86 | 76 | 77 | — | — | — | — | 0 32 | 0 41 | 0 78 | 0 58 | 0 52 | 5 15 | — | — |
| 10 | 91 | 78 | 87 | — | — | — | — | 0 30 | 0 63 | 0 46 | 0 62 | 1 14 | 2 34 | — | — |
| 11 | 75 | 75 | 76 | — | — | — | — | 0 70 | 0 67 | 1 13 | 0 78 | 0 88 | 4 66 | — | — |
| 12 | 91 | 92 | 75 | — | — | — | — | 0 63 | 0 53 | 1 13 | 0 84 | 0 73 | 5 85 | — | — |

plasma cholesterol 189±10 7 mg per 100 ml plasma ($n=12$) total phospholipids 187±12 2 mg per 100 ml plasma ($n=12$) and triglycerides 86±31 4 mg per 100 ml blood ($n=12$) During further fasting the levels of glucose lactate and pyruvate decreased slightly and the levels of free fatty acids aceto-acetate and β hydroxybutyrate increased (Table IV) Total cholesterol phospholipids and triglycerides remained essentially unchanged

DISCUSSION

As shown by Bjurulf (3) the subcutaneous fat tissue in the lower part of the abdomen is very homogenous consisting almost entirely of closely packed fat cells This is the reason why we have chosen the abdominal subcutaneous fat tissue for the comparison of fat tissue blood flow in different individuals

As a higher concentration of triglycerides in plasma might theoretically influence the partition coefficient value of xenon between fat tissue and blood a possible relationship between the calculated blood flow and the triglyceride level in plasma was searched for No such correlation was observed. Neither could the observed individual FBF differences be correlated to variations in blood hematocrit values

As earlier shown by Andree Larsen et al (1) the FBF is higher in lean than in obese subjects When the FBF values in the present study were plotted against abdominal skinfold thickness a similar inverse relationship was observed (Fig ?). The differences in FBF between diabetics and controls was however not correlated to differences in skinfold thickness No correlation was found between FBF in the diabetics and the duration of the disease or the age of the patients In the three patients with a known duration of the disease of less than one year without any clinically demonstrable signs of angiopathy or neuropathy the FBF was 11 0 8 6 and 23 9 ml/(100 g × min) values which are above the mean value for the controls

The mechanisms responsible for the high fat tissue blood flow in diabetics are not known One possible explanation might be an increased relative number of metabolically active units in diabetics However according to Bjurulf (3) diabetics show no decrease in the mean cell size in specimens of the subcutaneous fat, as might have been expected in the event of an increased number of cells per gram of tissue

It is a clinically well known phenomenon that patients with diabetic acidosis have an increased blood flow through the skin The present study indicates that this is the case also in subcutaneous fat tissue

The injection of small amounts of insulin which caused only slight changes in the blood glucose level and as expected a somewhat more marked lowering of the free fatty acid level caused on average a 50% decrease in the FBF Th pro

longation of the period of starvation from 10 to 17 hours on the other hand approximately doubled the FBF in the group of healthy controls More prolonged fasting which further altered the arterial levels of glucose free fatty acids and ketone bodies did not however further increase the FBF The abdominal skin temperature also increased significantly ($p < 0.01$) from the measurements at 10 hours to those at 17 hours of fasting but remained unchanged between 17 and 34 hours of fasting

These observations indicate that the FBF is dependent not only on external factors such as room temperature but also on factors related to the m tabolic state However in the present material there was no correlation between the FBF and *arterial* concentration of any of the substrates studied Further studies aimed to show whether variations in the uptake and oxidation of substrates in the adipose tissue influence the FBF are under progress in our laboratories (7)

## REFERENCES


1 Andree Larsen O Lassen N A & Quaade F Blood flow through human ad pose tissue determined with radioactive xenon. Acta physiol scand. 66 337 1966

2 Bergmeyer H U & Bernt F Enzymatische Bestimmung von Keton Korpern im Blut. Enzymol Biol Clin 5 65 1965

3 Bjurulf P Atherosc erosis and body bu ld, with special reference to size and number of subcutaneou fat cells Acta med scand Suppl. 166 1959

4 Duncombe W G The colorimetric microdetermination of long chain fatty acids. Biochem J 88 7 1963

5 Edwards D A W Hammond W H Healy M J R Tanner J M & Whitehouse R. H. Design and accura y of calipers for measuring subcutaneous tissue thickness Brit. J Nutr 9 133 1955

6 Hohorst H J L(+)laktat Bestimmung mit Laktat Dehydrogenase und DPN In Methoden der enzymatischen Analyse H U Bergmeyer ed Verlag Chemie Weinheim 1962

7 Haggendal E. Kerstell J Steen B & Svanborg A Blood flow and uptake of oxygen and substrates in forearm muscle and subcutaneous fat tissue in man Acta med scand 183 79 1968

8 Haggendal E Steen B & Svanborg A Measurement of blood flow through human abdominal subcutaneous fat tissue by local injection of radioactive xenon Acta med. scand 181 215 1967

9 Kety S S Measurement of regional circulation by the local clearance of radioactive sodium. Amer Heart J 38 321 1949

10 Lassen N A Muscle blood flow in normal man and in patients with intermittent claudication evaluated by simultaneous Xe 133 and Na24 clearance J clin Invest 43 1805 1964

11 Segal S Blair A E. & Wyngaarden J B An enzymatic spectro-photometric method for the determination of pyruvic acid in blood J Lab clin Med. 48 137 1956

12 Svanborg A & Vikrot O Plasma lipid fractions including individual phospholipids at various stages of pregnancy Acta med scand 178 615 1965

13 Walker P G A colorimetric method for the estimation of acetoacetate Biochem J 58 699 1954

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# URINARY EXCRETION OF SERUM PROTEINS IN RENAL DISEASE

## *Studies of Electrophoretic Fractions and IgA IgG and IgM*

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*Abstract* Proteins in serum and concentrated urine from 41 patients suffering from renal diseases have been examined by electrophoresis in cellulose acetate gel immunoelectrophoresis, and estimation of the immunoglobulin content. Clearance of electrophoretic fractions and immunoglobulins was assessed and each value was expressed as a proportion of the albumin clearance

Patients with pyelonephritis and polycystic kidneys excreted relatively more globulin than did patients with glomerulonephritis, diabetic nephropathy SLE, and renal amyloid. The difference was most marked in the case of β globulin, and immunoelectrophoretic analysis showed that this fraction in the first named patient group consisted almost exclusively of transferrin.

Immunoglobulin excretion showed no marked difference between the patient groups, with the exception that the urine of the pyelonephritis and the polycystic kidney patients always had a low content of IgA. Other variations could be attributed to variations in the total protein clearance

The presence of bacteriuria did not influence the composition of the urine protein. High clearance of protein was noted only in patients with relatively well preserved renal function In pyelonephritis the proteinuria was similar at different degrees of renal failure

The results are discussed with respect to the origin of proteinuria associated with renal tubular disease

Qualitative analysis of proteinuria has proved to be of great value in the diagnosis of renal diseases In glomerular damage the permeability to different proteins may vary and by estimating the clearance of proteins information on the morphological changes can be obtained (2 4 5 6 8 10) The protein analyses may thus be an important complement to the microscopical examination of renal biopsy specimens Protein analysis is also of diagnostic value in diseases which primarily involve the tubules (3 7 16 17 18) In these conditions the proteinuria is characterized by lower albumin concentration and relatively higher globulin concentration in comparison with the glomerular conditions and by the presence of proteins or lower molecular weight than albumin The origin of tubular proteinuria has not been made clear but it has been suggested that decreased reabsorption of the protein filtered off by the glomeruli would play a part (3 7) Another conceivable mechanism might be increased tubular secretion in inflammatory conditions It has also been suggested that the presence of urinary tract infection could give rise to increased excretion of ~ globulin in the urine (17)

In the present work proteins in serum and urine of 41 patients with various renal diseases have been studied by electrophoresis in cellulose acetate gel The object was to try to find out by analysis which features of protein excretion are characteristic of pyelonephritis and might be of diagnostic value The proteins were also studied by immunoelectrophoresis In view of the possible part played by the immunological mechanism in the causation of the proteinuria the contents of IgA IgG and IgM in serum and urine were estimated by the immunological technique The influence of a concurrent urinary tract infection and of renal function on the proteinuria pattern was also studied.

## MATERIAL

All the patients were inpatients at Karolinska sjukhuset. The distribution was as follows

*Pyelonephritis* ten cases, eight women and two men, mean age 53 years, microscopical examination (renal biopsy nephrectomy or autopsy) in six cases *glomerulonephritis* 13 cases, three women and ten men, mean age 39 years, microscopical examination in 1 cases,

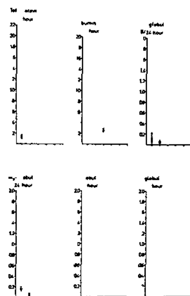


*Fig 1* Urine protein excretion divided into electrophoretic fractions Each point represents one patient *P* = pyelonephritis *G* = glomerulonephritis *D* = diabetic nephropathy *L* = systemic lupus erythematosus *A* = renal amyloid *C* = polycystic kidney

showing minimal changes in three membranous lobular glomerulonephritis in six and subchronic-chronic glomerulonephritis in three cases *diabetic nephropathy* six cases four women and two men mean age 55 years microscopical examination in one case *systemic lupus erythematosus (SLE)* in four cases all women mean age 36 years microscopical examination in one case *renal amyloid* five cases three women and two men mean ag 57 years microscopical examination of renal specimen in four cases and of rectal-biopsy specimen in one case *polycystic kidney* three cases all women mean age 62 years microscopical examination in two cases

## METHODS

Urine specimens were collected for 24 hours and stored at +4 C Serum samples were taken during the same 24-hour period Immediately after being collected 100–200 ml of the urine were concentrated by ultrafiltration under positive pressure of 7 kg per cm through a Diaflomembran UM 1 76 mm in diameter which retains particles of molecular weight > 10 000 (American Corp Cambridge Mass USA) Only urines that originally contained more than 0 5 g of protein per l were used and the concentration was carried on until the protein content was of the same order of magnitude as that in serum About 100 ml of urine were filtered in an hour

On ultrafiltration of urine the percentage distribution between the electrophoretic protein fractions is maintained, as is that between the immunoglobulins but the total protein loss is high (13) in the present study it was about 20% The quantity of the urine protein fractions was estimated on the basis of the protein content before concentration

Quantitative determination of protein was carried out by Kjeldahl s method for estimating nitrogen and the amount of protein was ascertained by multiplying by 6 25

*Electrophoresis* was carried out in strips of cellulose acetate gel (11) 2 5 × 15 cm in tris-veronal sodium buffer of pH 8 8 ionic strength 0 05 1 5 A per strip for one hour After separation the fractions were stained in a solution with Ponceau S stain The colour density was recorded with a Gelman scanner and subsequent planimetry (Gelman Instrument Co Ann Arbor Mich USA) This method gave good reproducibility as established by testing on normal sera and by duplicate determinations

Immunoelectrophoresis was done by Scheidegger s micromethod in veronal buffer of pH 8 6 ionic strength 0 01 using LKB 6800 A apparatus (LKB produkter AB Stockholm Sweden) Rabbit antisera to human serum proteins were used for analysis both of serum proteins and of urinary proteins

Immunoglobulins in serum and concentrated urine were estimated by the single radial diffusion method of Mancini et al (12) Commercial monospecific antisera against human serum IgA IgG and IgM were used Reference antigens were prepared as described elsewhere (14) All the specimens were analyzed at least twice

## RESULTS

### *Electrophoretic separation of urinary protein*

Fig 1 shows the absolute values for total protein and for the different fractions in each patient. With the exception of generally lower values in patients with pyelonephritis and polycystic kidneys there were no differences between the diagnostic groups

The values for the clearance of the fractions showed a wide range depending upon the degree of proteinuria When the clearance of each fraction was expressed as a proportion of the albumin clearance in the same patient (2) some characteristic features in the urine protein pattern were revealed This assessment of selectivity (5 8 9) was made in the following way

$$\left(\frac{UV}{P}\right)_{\text{globulin}} \Big/ \left(\frac{P}{UV}\right)_{\text{albumin}}$$

In patients with pyelonephritis or polycystic kidneys the clearance of $\alpha_2$ $\beta$ and $\gamma$ globulin in relation to that of albumin was higher than in patients with glomerulonephritis diabetic nephropathy SLE or renal amyloid (Fig 2) Exceptions were two patients with renal amyloid who also had relatively high excretion of $\beta$ and $\gamma$ globulin In both of them rheumatoid arthritis was the basis for amyloidosis none of them showed any signs

of urinary tract infection Selectivity values for β globulin seemed to be the most decisive feature as all the patients with pyelonephritis or polycystic kidneys had higher selectivity than any of the glomerulonephritis patients As regards the γ globulins on the other hand there was some overlapping Equally convincing differences between the patient groups were not obtained by comparing only the percentage distribution of the urinary protein fractions

*Immunoelectrophoresis*

Examination of urine concentrates from glomerulonephritis patients gave on the whole the same precipitin lines as those obtained with the corresponding serum An exception was the IgM line which could not be demonstrated in the urine Besides the distinct transferrin line at least two additional precipitates were observed in the β globulin region When urine concentrates from pyelonephritis patients were examined in the same way a distinct transferrin line predominated in the β globulin region (Fig 3) The same observation was made at examination of urine from a patient with polycystic kidneys



*Fig 3* Upper half shows immunoelectrophoresis of serum (*above*) and urine concentrate (*below*) from a patient with membranous glomerulonephritis Albumin fraction to the right. Lower half shows the same examination of serum (*above*) and urine concentrate (*below*) from a patient with pyelonephritis A distinct transferrin line predominates in the urine β globulin region In the glomerulonephritis urine at least three precipitates are seen in the β globulin region

*Immunoglobulins*

Immunoglobulin values in serum are shown in Fig 4 The normal values for the different immunoglobulins recorded in the figure are means from 370 clinically healthy control subjects published earlier (14) The glomerulonephritis patients had on an average low values for IgG One woman with pyelonephritis had an extremely high IgA value 1265 mg per 100 ml and one man with pyelonephritis had a very high IgG value 3020 mg per 100 ml In both cases the other immunoglobulins were only moderately raised None of the patients had any M-components

The urinalyses showed that the IgA excretion in pyelonephritis and polycystic kidney was low throughout (Fig 5) though some patients in the other disease groups had equally low values The IgG excretion varied independently of the di

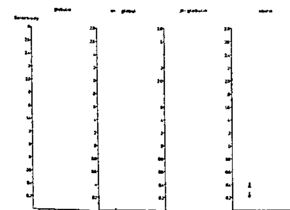


*Fig 2* Clearance of globulin fractions as a proportion of albumin clearance (= 1 0) For abbreviations see Fig 1

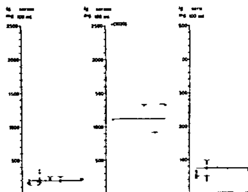


*Fig 4* Results of immunoglobulin analyses on serum. Mean ± S.D for healthy control subjects is indicated by lines in the picture Abbreviations as in Fig 1 Open circles indicate patients with bacteriuria

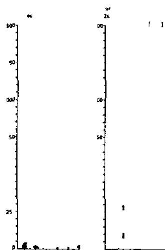


*Fig 5* Results of immunoglobulin analyses on urine Abbreviations and symbols as in Figs 1 and 4

agnosis The IgM content in urine was in most cases very low and will not therefore be reported in detail One patient with SLE and one with amyloidosis however excreted about 50 mg of IgM per 24 hours

When the urine was examined for its IgA con tent two precipitates were in most cases obtained with antiserum against plasma IgA The same phenomenon was never observed in the serum analyses For the quantitative determination the diameter of the outer precipitation disc was used This observation will be further considered in the discussion

The clearance values for immunoglobulins showed a wide range varying with the degree of proteinuria No differences typical of the various diagnoses were noted The same holds true for selectivity when the clearance of the globulins was compared with that of albumin (Fig 6)

The small number of patients representing different morphological changes in the glomerulonephritis group does not allow any conclusions as regards the relation between the microscopically observed glomerular damage and selectivity for excretion of immunoglobulins All three patients with minimal changes however had the lowest selectivity values for IgA in this group estimated as described in the foregoing This would indicate high selectivity of the protein excretion As regards IgG two of the patients had the lowest values in this group while one had a medium value

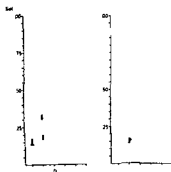


*Fig 6* Clearance of immunoglobulins in proportion to albumin clearance (1 0) Abbreviations as in Fig 1

*Influence of a concurrent urinary tract infection*

A concurrent infection refers here to the presence of significant bacteriuria diagnosed by culture immediately before the collection of urine for protein analysis The presence of an infection did not influence the different protein fractions in serum or urine nor their clearance or selectivity values The same was true of the immunoglobulins This will be seen from Figs 4 and 5 in which values for infected cases are indicated by open circles and for non infected cases by closed circles

*Influence of renal function*

The composition of the proteinuria as evident after electrophoretic analysis showed no variation referable to the degree of renal failure On the

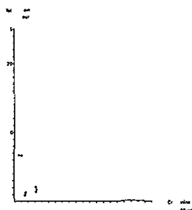


*Fig 7* Total excretion of protein relative to serum creatinine Open circles indicate patients with pyelonephritis closed circles patients with polycystic kidneys crosses other patients

other hand heavy proteinuria occurred only in patients with relatively well preserved renal function (Fig 7) This is consistent with the observation that in patients with the nephrotic syndrome the protein loss decreases with increasing renal failure In pyelonephritis patients the amount of protein in the urine was largely unchanged at varying degree of renal function

## DISCUSSION

The object of the investigation was to compare the proteinurea associated with an inflammatory disease involving primarily the tubules with the proteinurea that at least mainly is attributable to glomerular changes The latter form is represented not only by glomerulonephritis but also by diabetic nephropathy SLE and renal amyloid These patients might compensate for the skew distribution by age and sex when comparing the patients with pyelonephritis to those with glomerulonephritis

Electrophoretic studies of proteinuria have earlier been made to demonstrate the characteristic features of protein excretion in renal tubular damage The results were presented by description of the electrophoretic patterns The clearance of the electrophoretic protein fractions expressed in relation to albumin clearance have been estimated in permeability studies in cases of glomerular damage (2 9) No characteristic features correlated to the morphological changes could however be demonstrated The latter method was applied in the present study It has the advantage that any differences in clearance between albumin and globulin are brought out distinctly Further it is independent of urine volume and eliminates some sources of error such as variation of the dye binding properties of albumin and globulin or an excessively high globulin value owing to albumin lag since the value for each protein fraction is included both in the numerator and in the denominator (p 56) In the present study the selectivity measurements were found to give the most reliable information in revealing a difference between the proteinuria of glomerulonephritis and that of pyelonephritis

An increase in the clearance of $\beta$ globulin relative to albumin was most decisive in this respect This observation is in agreement with that found by other investigators namely that the $\beta$ globulin fraction in urine is markedly raised in pyelonephritis (16 20) the adult Fanconi syndrome (3) and tubular damage due to chronic cadmium poisoning (15) Immunoelectrophoresis showed the presence of a distinct transferrin line in pyelonephritis urine The observation differs from that made by others who found that the transferrin line was weak and atypical in these cases (16 20) This discrepancy in results can be solved by studying the transferrin clearance in various renal diseases

Monospecific antisera were used in measurements of immunoglobulins For quantitation we used standard immunoglobulin from serum The applied diffusion technique gives information on the presence of antigenically active components in the protein mixture which react with antibodies to the $\alpha$-chain in IgA and the -chain in IgG respectively that is the heavy chains No conclusions can be drawn as regards the presence of native protein or fragments The results reported for the immunoglobulins are based on the assumption that these consisted mainly of native protein

On analysis of IgA two precipitation arcs were obtained in most cases when urine concentrates were examined as against one with serum This indicates the presence of two antigenically very closely allied types of urinary IgA which differed from each other with respect to diffusion properties It might be assumed that the inner precipitation disc corresponded to the heavier IgA which has been demonstrated in normal urine (1) The obtained values referred to the outer precipitate which would thus correspond to serum IgA

The analyses of immunoglobulin in serum and urine gave no guidance for diagnosis The low IgG content in serum in glomerulonephritis paralleled a lowered $\gamma$ globulin level in this disease The high IgG content often seen in the urine paralleled a general protein leakage In pyelonephritis as well as in SLE very high serum immunoglobulin levels were seen in a few cases In pyelonephritis M-components can also be found in serum in rare cases This great variability should make it increasingly difficult to use the immunoglobulin analyses for diagnostic purposes in renal disease

That the variations in the immunoglobulin values were not due to the nature of the renal damage is further illustrated by the fact that the clearance and selectivity of these proteins did not vary markedly between the patient groups

The pyelonephritis patients as well as those with polycystic kidney had throughout low values for urinary IgA. They also had the highest IgG/IgA ratios in serum as well as in urine This cannot be explained at present The results suggested that in glomerulonephritis the use of selectivity studies of immunoglobulins should permit assessment of the nature of the glomerular damage as has been reported (4 5)

The presence of bacteriuria did not seem to affect the immunoglobulin clearance either in pyelonephritis or in other diseases This indicates that the infection per se does not influence the pattern of the proteinuria The determining factor would more likely be the cell damage caused by the infection

Thus, the present study has not provided any evidence for tubular secretion of protein into the urine due to bacterial infection. Reservation must be made for the possibility of entrance of secretory IgA from the urinary passages as this fraction was not measured It could not however constitute any quantitatively significant part of the urine proteins Such a secretion might be a link in the immunological defence against infection (19) The proteinuria of pyelonephritis resembles that of tubular damage of other origin, for instance poisoning (15) This supports the assumption that it acquires its pattern mainly from impaired reabsorption of protein from the primary urine

At varying degree of filtration the pyelonephritis patients had on the whole the same amounts of protein in their urines (Fig 7) and the composition was similar as far as electrophoretic fractions and immunoglobulin content were concerned These observations would seem to suggest a production process that is independent of filtration, i.e. secretion. An alternative explanation might be that the reabsorption decreases as the glomerular leakage of protein decreases owing to progressing tubular-cell damage impairment of function as a result of uraemia, osmotic diuresis or some other disturbance of glomerulo-tubular balance

## REFERENCES


1 Bienenstock, J & Tomasi, T B., Jr Secretory γA in normal urine. *J clin. Invest.* 47 1162, 1968
2 Blainey J D., Brewer D B Hardwicke J & Soothill, J F. The nephrotic syndrome Quart. J Med. 29 235 1960
3 Butler E. A. & Flynn, F V The proteinuria ... renal tubular disorders Lancet 2 978 19..
4 Cameron, J S Histology protein clearances ... *response to treatment in the nephrotic syn...* Brit. med. J 4 352 1968
5 Cameron, J S & Blandford, G. The simpl... ment of selectivity in heavy proteinuria. Lancet ... 1966
6 Cohen, A M & Walker W G The use of *clearances of enzymes as an indicator of ...* permeability of the renal glomerulus J Lab c... Med. 70 571 1967
7 Creeth J M., Kekwick, R. A., Flynn, F V Harris H & Robson, E. B An ultracentrifuge study of urine proteins with particular reference to the proteinuria of renal tubular disorders Clin. chim. Acta 8 406 1963
8 Hardwicke J & Soothill, J F. Glomerular damage in terms of "pore size" Ciba Symposium, Ren... Biopsy pp 32–42 Churchill, London 1961
9 Hardwicke J & Squire J R. The relationship between plasma albumin concentration and protein excretion in patients with proteinuria Clin. sci. 14 5.. 1955
10 Hardwicke J., Blainey J D., Brewer D B & Soothill, J F The nephrotic syndrome vol. 3 pp 69–.. Proc Third Int. Congr Nephrol., Washington 1966.
11 Kohn, J Cellulose acetate electrophoresis and immuno-diffusion techniques In. Chromatographic and electrophoretic techniques vol. II pp ..6–90 I. Smith, ed. Wm. Heinemann (Medical Books) Ltd., London 1960
12 Mancini, G., Carbonara, A. O & Heremans, J F Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry .. 1965
13 Miyasato F & Pollak, V E. Serum proteins in urine an examination of the effects of some methods used to concentrate the urine J Lab clin. Med. 6.. 1036 1966
14 Norberg, R. *The immunoglobulin content of normal serum.* Acta med scand. 181 485 1967
15 Piscator M Proteinuria in chronic cadmium poisoning. J environm. Hlth 4 607 196..
16 Revillard, J P Les protéinuries au cours des maladies renales Diss Gatheron, Lyons 1964
17 Tidstrom, B *Urinary protein in glomerulonephritis* and pyelonephritis. Electrophoretic analyses Acta med scand 174 385 1963
18 — Paper electrophoresis in the diagnosis of renal diseases. In Protides of the biological fluids, pp 311–319 H. Peeters ed. Elsevier Amsterdam, London and New York 1965
19 Tourville D R. Bienenstock, J & Tomasi, T B Jr Quoted in (1)
20 Traeger J., François R., Creyssel, R., Revillard, J P., Manuel, Y., Freycon, M. F & Sie M. Analyse électrophorétique des protéinuries rencontrées au cours des atteintes tubulaires rénales J Urol Néphrol. 1.. 7.8 1965

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# THE EFFECT OF THYROID HORMONES ON THE URINARY EXCRETION OF TAURINE IN MAN

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*Abstract* The urinary excretion of taurine has been determined for six hypothyroid patients before and after therapeutic treatment with thyroid hormones Three of the patients were fed a standardized diet in isocaloric amounts during both parts of the study whereas the other subjects were given the hospital diet without restriction Duodenal bile collected from three patients was analysed for bile acids After therapy the urinary taurine increased in all patients and the elevated ratio between glycine and taurine conjugated bile acids dropped towards a normal range Treatment with thyroid hormones did not influence the urinary excretion of sulphate in two of three patients studied It was concluded that the effect of treatment on the excretion of taurine in hypothyroid patients was not due to an elevated intake of sulphur amino acid with the diet.

A recent report of Foley et al (7) has demonstrated an association between hypothyroidism and a disturbance of the peripheral release of amino acids It appears from their paper that thyroid hormones in this respect have an unequal influence on different amino acids A similar tendency was observed concerning the concentration of amino acids in blood The arterial blood plasma concentration of $\alpha$ amino-$n$ butyric acid valine methionine isoleucine and leucine was lower in myxedematous subjects than in healthy subjects whereas no significant differences could be observed for other amino acids examined

Indirect evidence that thyroid hormones influence the metabolism of taurine has previously been obtained in connection with studies on the bile acids The human liver excretes the bile acids conjugated with glycine and taurine The normal ratio between glycine and taurine conjugated bile acids (G/T ratio) in human bile is approximately 3 2/1 (21) An increased conjugation with glycine has been a consistent finding in the bile of patients with hypothyroidism (9 10) Since the G/T ratio appears to vary with the amount of taurine available for conjugation (3 19) it seems likely that the changed pattern of bile acid conjugation in hypothyroidism is brought about by taurine deficiency

Taurine 2 aminosulphonic acid is one of the end products in the catabolic pathway of the sulphur-containing amino acids methionine and cysteine The dominating metabolite of these compounds is inorganic sulphate which is also the major sulphur fraction of the urine Most diets contain only small amounts of inorganic sulphate and a predominant part of this substance in the urine is derived from oxidation of methionine and cysteine (23) Studies with healthy subjects have shown that the urinary excretion of inorganic sulphate under certain conditions closely correlates with the amount of sulphur-containing amino acids in the diet (14 17)

In the present study we have endeavoured to obtain further information about the metabolism of taurine in hypothyroidism The urinary taurine excretion has been studied in myxedematous patients before and after some months of treatment with thyroid hormones In additional experiments methionine and cysteine have been administered to hypothyroid patients and healthy control subjects and the urinary excretion of inorganic sulphate has been assessed

## MATERIAL AND METHODS

The test material comprised two male patients (nos 4 and 8) and five female patients The age of the patients five of whom had primary hypothyroidism, varied from 47 to 78 years (mean 59 3 years) The control subjects were younger (34–36 years) one of them (no 11) was female

Table I *Urinary excretion of taurine (mmoles/24 h) before and after 1.5–3 months of treatment with thyroid hormones*

| Pat no | Diet | Before treatment | After treatment |
|---|---|---|---|
| 1 | Free | 0 37[a] ± 0 098 (6[b]) | 0 61 ± 0 100 (4) ($p$ < 0 01) |
| 2 | Free | 0 34 ± 0 199 (4) | 1 20 ± 0 236 (4) ($p$ < 0 001) |
| 3 | Free | 0 51 (0 45–0 57) (2) | 1 01 (0 29–1 45) (6) |
| 4 | Stand | 0 29 ± 0 030 (5) | 0 61 ± 0 101 (3) ($p$ < 0 001) |
| 5 | Stand | 0 12 (0 14–0 10) (*) | 0 29 (0 27–0 30) (2) |
| 6 | Stand. | 0 21 | 0 40 |

[a] Values are means ± S D
[b] Number of experiments

Patients 4 and 7 were thyroidectomized 6 weeks prior to the present study because of a thyroid carcinoma Each patient was examined during two experimental periods, before and after 2–5 months of treatment with thyroid hormones (thyroxine or desiccated thyroid preparations) During the first part of the study all patients exhibited unequivocal evidence of myxedema. The PBI ranged from 1 0 to 2.9 μg% (mean 1 4 μg%) and the serum cholesterol from 386–660 μg % (mean 501 μg%) The neck uptake of $^{131}$I at 24 h in the non-thyroidectomized patients was below 9% When re-examined after treatment the patients had lost their myxedematous features and were clinically normal. All patients were hospitalized during the study

Patients 1 2 and 3 were supplied with the regular hospital diet without restrictions whereas patients 4 5 and 6 were given a standardized diet in isocaloric amounts during both parts of the study This diet was of formula type and had been prepared of butter skimmed milk powder and water as described earlier (8) The patients were allowed to drink water tea and coffee freely and, in addition, they were given a fixed amount of white bread, marmalade and fruits. The caloric intake was fixed according to the subject s demands, and isocaloric amounts of the diet were given during both experimental periods

Collection of urine started when the patients had been fed the diet for 3–5 days. Urine was obtained in 24 h portions, aliquots of which were stored at −15 C until worked up Taurine was analyzed spectrophotometrically after being purified on a cation exchange column as described by Sorbo (22) Inorganic sulphate was determined by turbidimetric analyses (?) Bile samples were collected from patients 1 2 and 3 through a duodenal tube after intravenous injection of cholecystokinin-pancreozymin preparation (Cecekin® Vitrum) The bile acid separations were performed by quantitative paper chromatography (20)

Table II *Ratio between glycine and taurine conjugated bile acids before and after treatment with thyroid hormones*

| Pat. no | Before treatment | After treatment |
|---|---|---|
| 1 | 7 6 | 3 1 |
| 2 | 8 5 | 3 8 |
| 3 | 5 8 | 2 6 |
| Mean ± S.D | 7 3 ± 1 37 | 3 2 ± 0 60 ($p$ < 0 01) |

## RESULTS

The values obtained for the urinary excretion of taurine are given in Table I In the three hypothyroid patients who were given the free hospital diet the 24 h urinary taurine ranged between 0 34–0 51 mmoles (mean 0 41 mmoles) When the patients were re-examined after being treated with thyroid hormones for 3–5 months the excretion of taurine in the urine had increased in all subjects The mean value now obtained was 0 94 mmoles (range 0 61–1.20 mmoles) When expressed as percentage change of the valu s before therapy the increase of urinary taurine in subjects 1 2 and 3 was 65 252 and 95 respectively In agreement with previous observations in myxedematous patients the G/T ratio of the bile acids was elevated above normal during the first experimental period After treatment the G/T ratio

Table III *Urinary excretion of inorganic sulphate (mmoles/24 h) before and after 1.5–3 months of treatment with thyroid hormones*

| Pat no | Diet | Before treatment | After treatment |
|---|---|---|---|
| 1 | Free | 20 0 ± 11 30[a] (5)[b] | 18 7 ± 2 06 (4) ($p$ < 0 1) |
| 2 | Free | 12 3 ± 1 38 (4) | 16 0 ± 1 94 (4) ($p$ < 0 0 ) |
| 4 | Stand | 31 3 ± 7 88 (5) | 33 6 ± 3 99 (3) ($p$ < 0 1) |

[a] Values are means ± S D
[b] Number of experiments

Table IV *Inorganic sulphate in the urine (mmoles/24 h) before and during oral administration of cysteine or onine*

| Subject | | Control diet | Control diet – cysteine (3 g d) | Control diet + methionine (3 g d) |
|---|---|---|---|---|
| Pat no | | | | |
| 4 | I | 31 3 (18 6–35 0) | 36 2 | 36 9 |
| 4 | II[b] | 33 6 | 46 1 | 41 4 |
| 7 | | 15 9 (14 4–18 8) | 34 5 | 29 0 |
| 8 | | .4. (15 6– 8 1) | 45 2 | 37 5 |
| Control subjects | | | | |
| 9 | | 8 5 (.5 0–3 2) | 38 9 | 43 7 |
| 10 | | 30 8 (30 6–31 0) | 44 0 | 43 5 |
| 11 | | 24 2 ( 0 8–26 9) | 37 7 | 35 9 |

Before treatment with thyroid hormones
[b] After three months treatment with thyroid hormones

decreased to normal levels in all subjects (Table II)

Before as well as after therapy the patients fed the formula diet excreted less taurine than the patients who were given the free hospital diet (Table I) However the increase of the urinary taurine after treatment (90–142 %) was similar to that observed for the first group of patients

The urine of patients 1 2 and 4 was analyzed for the amount of sulphate (Table III) The results obtained for patients 1 and 4 before and after therapy did not differ whereas patient 2 (free hospital diet) excreted more sulphate while substituted with thyroid hormones

The last series of experiments included three control subjects and three of the hypothyroid patients All subjects were fed the formula diet After a running in period of 3–5 days the caloric intake was kept unchanged for the experimental period When four 24 h urinary portions had been collected the subjects were given 3 g of methionine for two days After an interval of one day cysteine (3 g) was administered during the next two days The administration of the amino acids resulted in an increased excretion of urinary sulphate in all subjects (Table IV) The net increase recorded for patients 7 and 8 after administration of methionine (13 1 and 13 3 mmoles) was similar to that of the control subjects (11 7–15 2 mmoles) When cysteine was given the amount of extra sulphate in the urine of patients 7 and 8 exceeded that of the control subjects

Patient 4 (formula diet) was examined according to the schedule outlined above both before and after three months of treatment with thyroid hormones The urinary excretion of sulphate during the two control periods was 31 3 and 33 6 mmoles respectively The administration of cysteine and methionine to this patient while myxedematous resulted only in a moderate increase of the urinary sulphate On the second occasion when the patient was euthyroid the sulphate excretion had increased to the level encountered in the control subjects The administration of methionine once again resulted only in a moderate (8 8 mmoles) extra excretion of urinary sulphate

The urinary excretion of taurine was higher in the three control subjects (0 55–1 16 mmoles day) than in the two hypothyroid patients 7 and 4 (0 13 and 0.29 mmoles/day respectively) When cysteine was added to the diet a slight increase of the urinary taurine was observed for subjects 7 9 and 10 This difference was not significant on a statistical basis

## DISCUSSION

Next to glycine taurine is the most important ninhydrin positive compound in adult human urine The excretion of taurine in the urine is influenced by deficiences of vitamins B and $B_1$ and by the administration of steroid hormones Hypertaurinuria has been observed in several diseases such as muscle disorders cirrhosis of the liver and in renal amino aciduria An increased tissue breakdown (as for instance after X ray radiation) results in an increased excretion of taurine in the urine and possibly in a slight elevation of the plasma taurine concentration (for an extensive review see (12))

The pattern of bile acid conjugation in human bile varies on a free diet (21) Feeding of taurine results in a marked decrease of the G T ratio (19) An abnormally low G T ratio has also been reported for patients with chronic liver disease which in addition may be accompanied by increased levels of taurine in plasma and urine (12) An increase in glycine conjugation has been ob-

served in cysteine deficient rats (6) and in rats fed diets deficient in vitamin $B_6$ (1 5)

It appears from the studies mentioned above that the urinary taurine under certain conditions reflects the amount of taurine available for conjugation of bile acids An increased excretion of taurine in the urine is accompanied by a low G/T ratio of the bile acids and vice versa The same relationships between taurine in bile and urine was observed in the present study of hyperthyroid patients Upon treatment with thyroid hormones the elevated G/T ratios decreased to normal values and the urinary excretion of taurine increased

A change of the taurine in bile and urine may or may not be accompanied by a change of the taurine in other tissues Rats deficient in pyridoxine have a low excretion of taurine in the urine and an elevated G/T ratio of the bile acids In these animals however the taurine content in brain liver spleen and muscle is reported to be normal (11 15) Thus it is not possible on the basis of the present data to make any statements about the effect of thyroid hormones on the taurine body pool However because of the enterohepatic circulation of the bile acids it seems possible that the pattern of bile acid conjugation might respond rapidly to changes of the taurine balance The human liver synthesis of bile acids is approximately 700 mg per day (13) In cases with a normal G/T ratio (3 2/l) the amount of taurine *required for conjugation should be approximately* 0 4 mmoles per day The daily need of taurine for conjugation should be higher Thus the total output of bile acids from the liver is approximately 20–30 g per day (4) During the enterohepatic circulation there is a considerable hydrolysis of the conjugated bile acids (4) Part of the taurine thus liberated is re absorbed whereas another part is converted to inorganic sulphate (12) The catabolism of taurine in the human intestines is insufficiently known The faecal excretion of taurine in man has been found to be approximately 20 mg per day (16)

The relative contributions of ingested and endogenous synthesized taurine to taurine pools in human tissues and body fluids are essentially unknown Taurine is synthesized endogenously and supplied with *the diet The normal intake in man* has been estimated at 40–400 mg p r day (16) A spontaneous reduction of the food intake may contribute to the lowering of taurine in bile and urine of hypothyroid patients The influence of the diet appears to be of minor importance since the increase of urinary taurine after treatment of thyroid hormones is similar whether the intake of sulphur amino acids is kept constant or not

An impaired absorption of the sulphur amino acids in the intestines may also b reflected by a reduction of taurine in bile and urine Methionine in normal subjects is absorbed predominantly in the proximal part of the small intestine (18) Within 24 h after oral administration of methionine and cysteine there is a marked increase of the urinary sulphate The net increase of the urinary sulphur (as sulphate) has been found almost to equal the amount of sulphur of the amino acids administered (14) In the present study methionine and cysteine were given to three hypothyroid and three normal subjects who were fed a standardized diet The very similar net increase of inorganic sulphate in the urine of two hypothyroid and the three control subjects may be taken as evidence for a normal absorption of the sulphur amino acids in hypothyreosis It seems possible then that factors other than those discussed above are responsible for the reduction of taurine in bile and urine of hypothyroid patients The findings of Foley et al (7) of low release of several amino acids from the tissues appear to be a feasible explanation

## REFERENCES


1 B rgeret B & Chatagner F Biochim biophys Acta (Amst) ?? 273 1956
2 Berglund F & Sorbo B Scand J clin Lab Invest 1? 147 1960
3 B rgstrom S & Gloor U Acta chem scand 9 34 1955
4 Bergstrom S Danielsson H & Samuelsson B Lipide metabolism pp 291–336 John Wiley & Sons, New York 1960
5 Doisy E A Jr Daniels M & Zimmerman S M A Fed Proc 15 243 1956
6 Eriksson S Unpublished observations cited in Lipid metabolism p 330 John Wiley & Sons New York 1960
7 Foley T H London D R & Prenton M A J clin Endocr 26 781 1966
8 Hellstrom K Acta physiol scand 63 .1 1965
9 Hellstrom K & Sjovall J J Atheroscler Res 1 ?05 1960
10 Hellstrom K & Lindstedt S J Lab clin Med 4 666 1964
11 Hope D B J Neurochem 1 364 1957

1 Jacobsen J G & Smith L H Jr Physiol Rev 48 4 4 1968
13 Lindstedt, S Acta physiol scand 11 417 1957
14 Medes G Biochem J 31 1330 1937
15 Nyffenberger E K, Lauber K & Aebi H Biochem Z *333* 226 1960
16 Roe D A J invest Derm 46 4 0 1966
17 Sabry Z I Shadarevian S Covan J W & Champell J A Nature ,06 931 1965
18 Schedl H Pierce C E Rider A & Clifton J A J clin Invest 47 417 1968
19 Sjovall J Proc Soc exp Biol (N Y) 100 676 1959
,0 — Clin chim Acta 4 652 1959
21 — Clin chim Acta 5 33 1960
?? Sorbo B Clin chim Acta 6 87 1961
?3 Young L & Maw G A Metabolism of sulphur compounds Methuen & Co London 1958

drug, 18 were found to have ANF and the increased frequency was ascribed to the use of the drug None of the patients had clinical symptoms or signs of SLE or of any other autoimmune disease It was therefore considered worth while to study a similar series to see if phenothiazines could induce ANF

## MATERIAL AND METHODS

*Clinical groups*

All female patients (488) of a mental hospital in a Swedish town (Östra sjukhuset in Malmö) with about 250 000 inhabitants were examined for ANF

The patients were divided into three diagnostic groups

Group 1 *Schizophrenia* (226 pats ) Most of these had chronic schizophrenia

Group 2 *Other mental diseases* (99 pats ) This was a heterogeneous group of patients with such diseases as manodepressive disease alcoholism certain organic conditions and some cases of psychopathy and severe neuroses

Group 3 *Senile dementia* (163 pats ) To this group were assigned particularly degenerative and vascular cases of pathological aging

Inpatients of a Swedish mental hospital constitute a very heterogeneous group The duration of hospitalisation varies It may be assumed that the schizophrenic group in particular consists of chronically ill hospitalized cases because newly detected cases are now treated at the Outpatient Department if this is possible A large proportion of the inpatients have geriatric diseases such as senile dementia and, as far as the present investigation is concerned this group must be distinguished from the others from which it differs regarding treatment and frequency of somatic diseases

*Age*

The median age of the patients in the schizophrenic group was 63 years (range 19–96) in the group with other mental diseases it was 55 years (range 14–80) and in the group with senile dementia it was 79 years (range 55–94)

*Psychopharmaceutic therapy*

In the treatment of patients in mental hospitals the drugs used are often varied Since chlorpromazine has proved relatively toxic the groups were divided with this in mind The time of treatment has been taken into account to some extent.

Subgroup 1 *Receiving chlorpromazine* To this subgroup were assigned all patients receiving treatment with chlorpromazine

Subgroup 2 *Previous treatment with chlorpromazine* Of the remaining patients those who had previously been treated with chlorpromazine were referred to this subgroup

Subgroup 3 *Treatment with other phenothiazines* Of the rest of patients those who were receiving some form of phenothiazine treatment other than chlorpromazine, e g. thioridazine levomepromazine trifluperazine and flufenazine were allotted to this subgroup

Subgroup 4 *Remainder* This subgroup consisted of patients who had not previously been treated with chlorpromazine and who were not receiving any form of phenothiazine treatment at the time of the investigation These patients were being treated with e g. tricyclic antidepressives thiaxanthene derivatives butyrophenone derivatives sedatives or no drugs at all

Subgroups 3 and 4 together constitute a category which may be called *Not treated with chlorpromazine*

It should be pointed out that the principles of treatment varied with the type of disease The group *Schizophrenia* described here was treated more intensively with psychopharmaceutics than the other groups with regard to number of patients, dose and time These patients had received the largest average total dose of chlorpromazine As to the group *Other mental diseases* the principles of treatment were less uniform but, as a rule the psychopharmaceutic treatment at the hospital in question is fairly intense It should however be stressed that in treatment of patients with senile dementia phenothiazine is usually given in only small sedative or somnifacient doses In this respect the group *Senile dementia* thus differs essentially from the other two groups In Table I the patients are arranged according to diagnosis and treatment.

The group *Previous treatment with chlorpromazine* was separated off and studied for the reversibility of the ANF reaction.

Weller and Coons (19) *indirect fluorescent antibody technique* as modified by Hijmans et al (14) was used in the examination for ANF

The investigation was performed as a double blind test Known positive and negative sera were included as controls Human leucocytes from healthy individuals with blood group O were used as a substrate for binding ANF Blood films were treated with the patient's serum undiluted and with a commercial fluorescent conjugate [anti-human globulin (horse origin) fluorescein conjugated (Progressive Laboratories Inc B ltimore available through Roboz Surgical Instrumental Co)] diluted 1/16 containing antibodies to human IgG IgM and IgA The degree of nuclear fluorescence in positive preparations was graded from + to + + + + In + + + + preparations the nuclei showed a peripheral strong fluorescence In + + + to + preparations the entire nuclei showed a decreasing scale of fluorescence Preparations with fluorescent material outside the cell membrane only but so arranged as to suggest nuclear origin were designated (+) The ANF-positive material of grade + + + to + was not studied with regard to the pattern of fluorescence

A Leitz fluorescence microscope with a Philips Cs 150 W lamp an UG 1 primary filter and an ultraviolet absorbing secondary filter were used for examining the specimens

Tables I and II give the distribution of ANF among

Table I *Distribution of ANF in different diagnostic and treatment groups*

| Diagnosis/treatment | ANF A | B | C | D | E | F | Total A–F | Total D–F | D–F |
|---|---|---|---|---|---|---|---|---|---|
| Schizophrenia | | | | | | | | | |
| Receiving chlorpromazine | 81 | [illegible]0 | 3 | 17 | 21 | — | 14 | 38 | [illegible]6 8 |
| Previous treatment with chlorpromazine | 60 | 3 | 3 | 5 | 3 | 1 | 75 | 9 | 12.0 |
| Treatment with other phenothiazines | 4 | — | — | — | — | — | 4 | — | — |
| Remainder | 4 | 1 | — | — | — | — | 5 | — | — |
| Total | 149 | 4 | 6 | 22 | [illegible]4 | 1 | 2 6 | 47 | [illegible]0 8 |
| Other mental diseases | | | | | | | | | |
| Receiving chlorpromazine | 22 | 4 | 1 | 3 | 4 | — | 34 | 7 | [illegible]0 6 |
| Previous treatment with chlorpromazine | 26 | 2 | — | 4 | — | — | 32 | 4 | 12.5 |
| Treatment with other phenothiazines | 16 | 2 | — | — | — | — | 18 | — | — |
| Remainder | 12 | 1 | 1 | 1 | — | — | 15 | 1 | 6 7 |
| Total | 76 | 9 | 2 | 8 | 4 | — | 99 | 12 | 1 1 |
| Senile dementia | | | | | | | | | |
| Receiving chlorpromazine | 30 | 4 | — | 5 | 3 | — | 42 | 8 | 19 0 |
| Previous treatment with chlorpromazine | 25 | 4 | 1 | 3 | 1 | — | 34 | 4 | 11 8 |
| Treatment with other phenothiazines | 37 | 1 | 3 | 1 | 1 | — | 43 | 2 | 4 7 |
| Remainder | 38 | 2 | — | 2 | 2 | — | 44 | 4 | 9 1 |
| Total | 130 | 11 | 4 | 11 | 7 | — | 163 | 18 | 11 0 |

Grade of fluorescence of ANF
A −
B (+)
C (+) or +
D + or ++ ++ ⎫
E ++ or +++ +++ ⎬ ANF clearly positive
F +++ or ++++ ++++ ⎭

the subgroups Only those cases with fluorescence of at least grade + or ++ (subgroups D E, F in Table I) were accepted as ANF positive in the statistical analysis. For the sake of completion other ANF (categories B and C) are accounted for in Table I.

The reliability of the method for determining ANF was assessed by freezing 50 negative and 94 positive coded sera The correlation (intraclass correlation = $R$) between the original measurements and those after storage was $R = 0\ 57$ for the positive cases The magnitude and direction of the change were analysed As expected storage often resulted in a reduction of the antibody titer Such reduction which was noted in 73 of 94 cases, was statistically significant (the Wilcoxon matched pairs signed ranks test, Siegel 1956 $Z = 6\ 16$ $p < 0\ 001$) All negative tests proved negative also at re testing and none of the positive tests turned negative or doubtful The result suggested that ANF as measured by the method used are constant enough to allow the use of stored frozen serum samples The coefficient of correlation between measurements made by two examiners was $R = 0\ 78$ The corresponding values for the respective examiners were $R = 0\ 89$ and $R = 0\ 79$ The reliability of the method as judged by parallel measurements, was thus high and sufficient to characterise groups

## RESULTS

Subgroups 3 and 4 were small when grouping was done according to diagnosis (Table I) Therefore to permit statistical analysis they were pooled and the resulting group called *Not treated with chlorpromazine* As mentioned in the description of the method ANF was said to be present only when the reaction was clearly positive i e at least + or ++

The frequency of ANF in the three diagnostic groups is given in Table I ANF were found in altogether 20 8% of the group *Schizophrenia* in 12 1% of *Other mental diseases* and in 11 0% of *Senile dementia* If only the groups *Schizophrenia* and *Other mental diseases* which resemble one another regarding the treatment given are considered the frequency of ANF in the subgroups (Table II) will be 26 0% for *Receiving chlorpromazine* 11 3% for *Previous treatment with chlorpromazine* 0 0 for *Treatment with other phenothiazines* and 5 0 for *Remainder*

The distribution of ANF among the various treatment subgroups of *Senile dementia* resembled that in the other diagnostic groups viz. 19 0% 11 8% 4 7% and 9 1% respectively

Since the senile dementia group differed essentially from the other two diagnostic groups regarding principles of treatment the following statistical analysis was based only on the diagnostic groups *Schizophrenia* and *Other mental diseases* The analysis showed that ANF occurred more

Table II *Frequency of ANF in the diagnostic groups Schizophrenia and Other mental diseases related to treatment*

| | Receiving chlorpromazine | | Previous treatment with chlorpromazine | | Treatment with other phenothiazines | | Remainder | | Total |
|---|---|---|---|---|---|---|---|---|---|
| Negative | | | | | | | | | |
| A − | 103 | (58.2) | 86 | (81.1ᵃ) | 20 | (90.9) | 16 | (80.0) | 225 |
| Weakly positive | | | | | | | | | |
| B (+) | 24 | (15.8) | 5 | (7.6) | 2 | (9.1) | 2 | (15.0) | 33 |
| C (+) or + + | 4 | | 3 | | 0 | | 1 | | 8 |
| Clearly positive | | | | | | | | | |
| D + or + + + + | 20 | (26.0) | 9 | (11.3) | 0 | (0.0) | 1 | (5.0) | 30 |
| E + + or + + + + + + | 25 | | 3 | | 0 | | 0 | | 28 |
| F + + + or + + + + + + + + | 1 | | 0 | | 0 | | 0 | | 1 |
| Total | 177 | | 106 | | 22 | | 20 | | 325 |

often in the group *Schizophrenia* than in *Other mental diseases* This difference was however not significant ($\chi^2 = 3.48$, df 1 $p < 0.10$ two tailed test) On the other hand there was a statistically significant difference between the therapeutic groups ($\chi^2 = 16.13$ df 2 $p < 0.001$) Most of the cases with ANF were found in the group *Receiving chlorpromazine* and least often in the *Not treated with chlorpromazine* A correlation was found between ANF and treatment with chlorpromazine But since there was also a tendency for ANF to be associated with schizophrenia and since the frequency of patients treated with chlorpromazine was highest in the schizophrenic group (see Table I) the two diagnostic groups were compared for any difference with the treatment unchanged

No statistically significant difference was found between the two diagnostic groups

In the continued analysis the groups *Schizophrenia* and *Other mental diseases* were pooled On comparison between pairs of the various treatment groups the following differences were found

| | $\chi^2$ | df | $p$ (one tailed) |
|---|---|---|---|
| Receiving chlorpromazine/previous treatment with chlorpromazine | 7.35 | 1 | 0.005 |
| Receiving chlorpromazine/not treated with chlorpromazine | 10.95 | 1 | 0.001 |
| Previous treatment with chlorpromazine/not treated with chlorpromazine | 3.41 | 1 | 0.05 |

In the group *Receiving chlorpromazine* ANF proved statistically significantly more common than in the other two groups *Previous treatment with chlorpromazine* and *Not treated with chlorpromazine* ANF was also more common in the group that had previously received chlorpromazine than in the group that had not

To find out whether the age factor could explain the occurrence of ANF the frequency of ANF and the ages of the patients in each diagnostic group were studied for any correlation with the aid of four field tables No statistically significant relation was found It should however be mentioned that ANF was demonstrated in seven of the 129 patients who did not receive chlorpromazine Six of these patients were old but one was a woman aged only 28 years

*Clinical examinations and laboratory studies*

Seventy seven female patients with a clearly positive ANF reaction were examined further both clinically and by laboratory methods mainly for autoimmune disease (groups D E and F in Table I) Historical data were obtained mainly from the patients hospital records which may have been incomplete regarding somatic diseases If the patients had been treated at other departments their records were procured Further their records were studied for previous laboratory tests and other examinations Finally all patients with the exception of a few who had either died or moved out of town were examined clinically and with laboratory tests by us The clinical examination was performed by one of the authors (S B) The laboratory studies included determination of the ESR hemo-

globin concentration red blood cell count white blood cell count differential count platelet count reticulocyte count examination of the urine for albumin and glucose bilirubin urobilinogen urobilin and of the serum for glutamic acid/oxalacetic acid transaminase (GOT) glutamic acid/pyruvic acid transaminase (GPT) lactic acid dehydrogenase (LDH) bilirubin thymol reaction alkaline phosphatase electrophoresis serum tests for syphilis (STS) (Wassermann s Meinicke s and Kline s reactions) and for RA (including tests with sensitised sheep cells and acrylic plastics)

For practical reasons the series was divided into three categories

1 *physically healthy (laboratory tests normal)*
2 *patients with slightly increased GOT and/or GPT* and
3 *patients in whom the laboratory tests showed various abnormalities*

*Category 1*

Of 77 patients with a clearly positive ANF reaction the results of all the other laboratory studies were otherwise normal in 32 (41 6° ) None of the latter showed signs of autoimmune disease All 32 patients except four were being or had previously been treated with chlorpromazine

*Category 2*

Of the 77 patients GOT and/or GPT were increased in 18 (23 4 °) In two patients the gammaglobulin concentration was increased but in neither were any other signs of autoimmune disease demonstrable One patient had clinical signs of rheumatoid arthritis including a positive RA test Two clinically healthy patients had atoxic goiter which was not investigated further In one of these also the RA test was positive the other had previously had two attacks of chlorpromazine induced jaundice but tolerated chlorpromazine at the time of the investigation and appeared clinically healthy Three patients with a positive RA test appeared clinically healthy

*Category 3*

This category consisted of 27 patients (35 1 %) in whom laboratory studies had revealed various abnormalities It included three patients with serum M-components in a concentration of 0 2–0 5 g/100 ml The cause of this increase was not investigated further One of these three died from pulmonary embolism and bronchopneumonia and at autopsy sarcoidosis was suspected because of granulomas with giant cells in the lung One of the other patients with M-component had seven years previously been treated with radioactive phosphorous because of mycosis fungoids and serum electrophoresis 6 years later revealed an M-component in a concentration of 0 5 g/100 ml Twelve patients in this group had a positive RA test In one of them the serum gammaglobulin was 1 6 g/100 ml and clinical signs of rheumatoid arthritis as well as bilateral scleritic scars were noted One patient aged 69 had a previously known increased ESR hypergammaglobulinemia (2 8 g/100 ml) hypertension proteinuria anemia chronic biologic false positive reaction for syphilis (BFP) positive RA test and ANF but no demonstrable LE-cells Autoimmune disease probably of latent SLE type was assumed The patient died after a period of vomiting loss of body weight fatigue and fever Postmortem examination showed gross infarctions of the right kidney signs of chronic pyelonephritis of the left encephalomalacia and aortic aneurysm Microscopically lymphocytes and plasma cells were demonstrated in the left kidney and in the aortic media and adventitia One patient had folic acid deficiency anemia which responded to treatment with folic acid Finally one patient had previously been regarded as having a chronic BFP reaction though serological tests for syphilis at the time of our investigation were negative For several years serological tests for syphilis had been positive but the TPI test had been negative and she was now regarded by us as having a chronic BFP reaction At the investigation the ESR was moderately raised and there were signs of urinary tract infection but she was otherwise healthy

Seventeen (22 1 ) of the 77 patients had a positive RA test 12 (15 6° ) had hypergammaglobulinemia mostly of moderate severity and four (5 2° ) all aged had serum M-components The M components were of immunologic type G A and M and were of low concentration 0 2–0 5 g 100 ml except in one case where it was 1 3 g 100 ml They were not studied further for myelomatosis but they appeared to be clinically healthy

Table III. Forty-five patients with ANF showing various laboratory ab

| Pat no | Age (y) | ANF | Never chlorpromazine | GOT and/or GPT slightly increased | LDH increased | Bil/s increased | Alk phosph increased | Slight leukopenia | STS pos | RA test pos | Hyper gamma glob | M component | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 33 | 69 | E | | + | | | | | | | +2.2 g | | |
| 34 | 77 | D | + | + | + | + | | | | | +2.2 g | | Slight decompensation |
| 35 | 72 | E | | + | | | | | | + | | | Cholecystolithiasis |
| 36 | 43 | D | | + | | | | | | + | | | Atoxic goiter |
| 37 | 75 | E | + | + | | | | | | + | | | |
| 38 | 51 | E | | + | | | | + | | + | | | |
| 39 | 82 | E | + | + | | | | | | + | | +1.3 g | Rheum arthritis |
| 40 | 57 | E | + | + | | | + | | | | | | |
| 41 | 61 | E | | + | | | | + | | | | | |
| 42 | 74 | D | + | + | + | | | | | | | | |
| 43 | 77 | D | | + | | | | | | | | | Diabetes |
| 44 | 60 | E | | + | | | | | | | | | |
| 45 | 47 | D | | + | | | | | | | | | |
| 46 | 47 | E | | + | | | | | | | | | Atoxic goiter Had 2 bouts of chlorpromazine induced jaundice |
| 47 | 60 | E | | + | | | | | | | | | |
| 48 | 61 | E | | + | | | | | | | | | |
| 49 | 75 | L | + | + | | | | | | | | | |
| 50 | 58 | D | + | + | | | | | | | | | |
| 51 | 44 | L | | | | | | + | | | | | |
| 52 | 82 | D | | | | | | + | | | | | |
| 53 | 61 | D | + | | | | | + | | | | | |
| 54 | 59 | D | | | | | | + | | | | | |
| 55 | 45 | D | | | + | | | | | | | | |
| 56 | 71 | E | | | | | | | | | | | Epilepsy |
| 57 | 73 | D | | | + | | | | | | | +0.5 g | $^{32}$P treated mycosis fungoids |
| 58 | 85 | D | | | | | | | | | | +0.2 g | |
| 59 | 63 | E | | | | | | | | | | +0.5 g | Pulmonary sarcoidosis? |
| 60 | 79 | E | | | | | | | | + | | | |
| 61 | 67 | E | | | | | | | | + | | | |
| 62 | 67 | D | | | | | | | | + | | | |
| 63 | 71 | D | | | | | | | | + | | | |
| 64 | 66 | D | | | | | | | | + | | | |
| 65 | 68 | D | | | | | | | | + | | | |
| 66 | 71 | D | | | | | | | | + | | | |
| 67 | 59 | D | | | | | | | | + | | | |
| 68 | 67 | L | | | | | | | | + | | | |
| 69 | 63 | D | | | | | | | | + | +1.6 g | | |
| 70 | 69 | D | | | | | | | | + | +1.6 g | | Rheum arthritis with sclerotic scars |
| 71 | 57 | D | + | | | | | | + | + | +2.8 g | | Latent SLE Chronic BFP reactor |
| 72 | 71 | L | | | | | | | | | +1.5 g | | |
| 73 | 84 | E | + | | | | | | | | +1.4 g | | |
| 74 | 69 | E | | | | | | | | | +1.4 g | | Anemia due to lack of folic acid |
| 75 | 75 | E | | | | | | | | | +1.9 g | | Slight diabetes |
| 76 | 71 | D | | | | | | + | | | +1.3 g | | Epilepsy |
| 77 | [illegible] | [illegible] | | | | | | | | | [illegible] | | [illegible] |

Table III gives a list of the 45 patients with ANF and with other laboratory and serological abnormalities

## DISCUSSION

In our analysis of the material we included no cases with a weak ANF reaction because we have not been certain about the significance of such a finding It should however be mentioned that in a normal series consisting of 401 healthy volunteers mainly young blood donors (64 women and 337 men) only one per cent (1 woman and 4 men) had ANF These ANF reactions were all only weakly positive The method of analysis used was the same as that applied in the present investigation In our series the frequency of weakly positive ANF was substantially higher

Thus even if we consider only clearly positive ANF the frequency of ANF in our series of patients from a mental hospital was high The frequency of ANF clearly varied with the treatment with chlorpromazine In the group with schizophrenia which it may be assumed had received the largest total dose of chlorpromazine as many as 26 8 % of those who were receiving chlorpromazine at the time of the investigation had ANF In the other diagnostic groups the frequency of ANF during treatment with chlorpromazine was 19 0–20 6 % In the group not treated with chlorpromazine the frequency of ANF was 0 0–9 1 % The results show an unequivocal association between ANF and chlorpromazine ANF can with statistical certainty be ascribed to the chlorpromazine treatment This association though weaker was found to hold also for ANF and previous chlorpromazine treatment This lends strong support to the hypothesis that chlorpromazine favours the development of ANF

In our series we tried to find out whether treatment not only with chlorpromazine but also with other phenothiazine derivatives can induce the development of ANF In the groups *Schizophrenia* and *Other mental diseases* there were 22 patients treated with phenothiazine derivatives other than chlorpromazine In none of these was ANF demonstrable The treatment of this group was not uniform The 22 patients had received different phenothiazine derivatives These results thus indicated that ANF in our series should be ascribed to treatment with chlorpromazine while other phenothiazine derivatives appear not to have such a toxic effect Accordingly the phenothiazine skeleton is not by itself responsible for the ANF reaction This does not of course exclude the possibility of phenothiazine derivatives other than chlorpromazine being able to cause such a reaction Since the number of patients treated with different phenothiazine preparations was small the results should be interpreted with caution

In one group here called the *Remainder* the patients were treated mainly with tricyclic antidepressives thiaxanthene derivatives butyrophenon derivatives and sedatives In this group which consisted of 20 patients a positive ANF reaction was demonstrated with certainty in only one namely a 28 year-old woman who had received meprobamate

Of the group *Senile dementia* 19 % of those who were receiving chlorpromazine and 11 8 % of those who had previously received this drug though only in small doses had ANF Of the 87 patients who had not received chlorpromazine a positive ANF reaction was observed in six which must be regarded as a remarkably large number Of altogether 129 patients who had not received chlorpromazine ANF was demonstrated in seven Six of these were aged Only one with clearly positive ANF was below 67 years There were 32 patients below 67 years a relatively small group These differences suggest that advancing age might be able to explain at least some of the cases of ANF in our series In the schizophrenic group however no statistically significant correlation was found between the frequency of ANF and age and thereby indicates that it is the treatment per se that is responsible for ANF

Especially since autoimmune processes have been discussed as possible etiological factors of schizophrenia (10 11 12) an important question in this conjunction is whether schizophrenia is an important contributory causal factor of ANF We analysed our material for any covariation between ANF and schizophrenia By standardising treatment it was possible to detect differences if any between the diagnostic groups We found no statistically significant differences The investigation thus produced no evidence of any association between schizophrenia and ANF The tendency of the frequency of ANF to be higher in the schizophrenia group may be ascribed to the

treatment i e chlorpromazine treatment was more common in the schizophrenia group than in the others

One group that was separated off from the rest was the patients who had previously received chlorpromazine This treatment had thus been concluded before examination for ANF In these patients the frequency (11 3 %) of ANF was higher than in the groups that had never received chlor promazine (0 0–5 0 %) but lower than in the group that was still receiving chlorpromazine (26 0 %) This difference suggests that ANF due to treatment with chlorpromazine disappears on withdrawal of the drug

In the clinical and laboratory studies of the 77 women with ANF a search was made for auto immune diseases but no increased frequency of such conditions was found Two patients with rheumatoid arthritis including one who had been treated with chlorpromazine had ANF This fre quency is not remarkable because the disease is common especially among women On the other hand the occurrences of two patients without a definite diagnosis of autoimmune disease but with a chronic BFP reaction was more remarkable because this reaction should normally not occur (17) This points to a predisposition to autoim ne disease

In many patients the transaminases were also slightly increased sometimes associated with a low WBC count Such findings are not uncommon in patients treated with phenothiazines but the under lying mechanism will not be discussed here It is possible that also the increase of gammaglobu lins was due to administration of phenothiazines it being known that phenothiazines can produce early cirrhosis of the liver with an increase of the gammaglobulins (3 6 15)

22 1 % of the 77 patients had a positive RA test (positive acrylic plastics and/or positive sheep blood cell reaction) The frequency of rheumatoid factor however increases with age and Heimer et al (13) found a frequency of 46 % in elderly patients The frequency of a positive RA test in our series consisting mainly of old people was therefore not remarkably high

The frequency of M components in our series was 5 2 % In large normal series consisting of elderly people it is reported to be about 3 % (1 16) Our material was too small to allow any conclusion in this respect

Summing up there was no increased frequency of autoimmune disease among the above men tioned 77 patients but the possibility in two cases of a predisposition to autoimmune disease could not be excluded Treatment with chlorpromazine like methyldopa and isoniazid proved capable of causing antinuclear factors to appear in the serum There may be other preparations with a similar effect Since it is not possible to exclude a late development of autoimmune disease in these pa tients regular follow up is recommended par ticularly for antinuclear factors Should ANF or other serological abnormalities appear one should seriously consider withdrawal of the above men tioned drugs

## ACKNOWLEDGEMENTS

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## REFERENCES


1 Axelsson U Bachmann R & Hallén J Acta med scand 179 235 1966
2 Berglund S & Carlsson M Acta med scand 180 407 1966
3 Bloom J B Davis N & Wecht C H Amer J Psychiat 121 788 1965
4 Breckenridge A Dollery C T Worlledge S M Holborow E J & Johnson G D Lancet 2 1 65 1967
5 Cannat A & Seligmann M Lancet 1 185 1966
6 Danhof I E Tex Rep Biol Med 16 443 1958
7 Doniach D Roitt I M & Taylor K B Brit med J 1 1374 1963
8 Faurbye A & Clausen J Personal communication 1967
9 Haserick J R J chron Dis 1 317 1955
10 Heath R G & Krupp I M Arch gen Psychiat 16 1 1967
11 Heath R G Krupp I M Byers L W & Lilje kvist J S Arch gen Psychiat 16 10 1967
12 — Arch gen Psychiat 16 24 1967
13 Heimer R Levin F M & Rudd E Amer J Med 35 175 1963
14 Hijmans W Schuit H R E de Leeuw B & Mol ler H F Ned T Geneesk 106 2097 1962
15 Hollister L E & Hall R A Amer J Psychiat 123 211 1966
16 Hallén J Acta med scand Suppl 462 1966
17 Mulhern L M Masi A T & Shulman L E Lancet 2 508 1966
18 Seligmann M Cannat A & Hamard M Ann N Y Acad Sci 124 816 1965
19 Weller T H & Coons A H Proc Soc exp Biol (N Y) 86 789 1954

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# VARIATION IN PACING THRESHOLD

## *A Study in Patients with External Pacemaker and Unipolar Endocardial Electrode*

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*Abstract* The pacing threshold has been examined in patients with endocardial pacemaker electrode and external pacemaker A mean increase in pacing threshold was observed in the first 7 to 14 days after insertion of the electrode from 1 3 volt at the time of insertion to 2.5 volts after 7 days and 2 8 volts after 14 days In some patients an initial and transitory decrease was found in the first few days. Great individual variations were seen. Because of these variations it is in our opinion, necessary to check the threshold value in patients with myocardial infarction by daily measurements to adjust the pacemaker output at the lowest possible level Significant variations during the day and after meals sleeping or physical activity as reported by others, were not found.

The pacing threshold is the minimal electrical current necessary for initiating myocardial contraction in patients treated with artificial pacemaker The pacing threshold is dependent on the myocardial excitability (8) the impulse form and duration (1 2 6) the electrode area (3) the position of the endocardial electrode and the total impedance between the electrodes (8) It may vary considerably from patient to patient The pacing threshold is reported to increase the first days after insertion of the electrode (1 8 9) and to show variations during the day (9) in connection with meals (7 9) sleeping physical activity and drug administration (7 8)

In practical work spontaneous variations of the threshold value are of especial importance in the pacemaker treatment of patients with acute myocardial infarction In these patients it is desirable to use a lowest possible pacemaker output to diminish the risk of inducing ventricular arrythmias

The purpose of the present study was to examine to what extent it was necessary to take variations in pacing threshold into consideration in daily routine work

## MATERIAL AND METHODS

Twenty five patients with chronic A–V block and 17 patients with acute myocardial infarction and temporary pacemaker treatment for A–V block were examined Details concerning the patients are published elsewhere (4 ) Day to day variations in pacing threshold were studied in the 42 patients for 5 to 76 days after positioning of the endocardial electrode Spontaneous threshold variations during a 24 hour period were examined in nine of the patients, and threshold variations in relation to meals, sleeping and physical activity were examined in ten patients All the examinations were performed from 1 to 17 days after electrode insertion

Unipolar Elema EMT 588 electrodes were used in all patients A modified Elema 138 pacemaker in which rate and voltage could be gradually changed was used for threshold determinations. The pacemaker gave impulses of condenser-discharge type with a pulse duration of 3 0 milliseconds Pacing threshold was defined as the lowest voltage at which all impulses resulted in myocardial contraction when the pacemaker output was gradually increased The reading was possible with an accuracy of 0 1 volt.

The daily checks of pacing threshold were usually made between 10 and 12 a m In 17 of the patients the positioning of the electrode was guided by TV monitoring, and in 25 patients by ECG monitoring as previously described (5)

In order to examine the effect of meals, several measurements were made just before dinner and during the first hour after the meal In the study of the effect of sleeping measurements were made between 9 and 10 p m when the patients were still awake and about midnight while the patients were asleep The influence of physical activity on pacing threshold was examined by measurements before immediately after and 5 min after a light exercise in bed (the patients sat up and lay down 10 times at the speed of their own choice)

Patients in whom a range in pacemaker output of more than 0 4 volt from partial capture to full capture

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One group that was separated off from the rest was the patients who had previously received chlorpromazine This treatment had thus been concluded before examination for ANF In these patients the frequency (11 3 %) of ANF was higher than in the group that had never received chlor promazine (0 0–5 0 %) but lower than in the group that was still receiving chlorpromazine (26 0 %) This difference suggests that ANF due to treatment with chlorpromazine disappears on withdrawal of the drug

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In many patients the transaminases were also slightly increased sometimes associated with a low WBC count Such findings are not uncommon in patients treated with phenothiazines but the under lying mechanism will not be discussed here It is possible that also the increase of gammaglobu lins was due to administration of phenothiazines it being known that phenothiazines can produce early cirrhosis of the liver with an increase of the gammaglobulins (3 6 15)

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## REFERENCES

1 Axelsson, U Bachmann, R. & Hallén, J Acta med. scand 179 235 1966
2 Berglund S & Carlsson M Acta med scand. 180 407 1966
3 Bloom, J B., Davis N & Wecht, C H Amer J Psychiat. 121 788 1965
4 Breckenridge A Dollery C T Worlledge S M Holborow E. J & Johnson G D Lancet 2 1265 1967
5 Cannat A. & Seligmann, M Lancet 1 185 1966
6 Danhof I E. Tex. Rep Biol. Med 16 443 1958
7 Doniach D Roitt I. M & Taylor K. B Brit. med. J 1 1374 1963
8 Faurbye A. & Clausen, J Personal communication, 1967
9 Haserick, J R. J chron. Dis 1 317 1955
10 Heath, R. G & Krupp I. M Arch. gen Psychiat. 16 1 1967
11 Heath, R G Krupp I. M Byers L W & Lilje kvist J S Arch. gen. Psychiat 16 10 1967
12. — Arch gen. Psychiat 16 24 1967
13 Heimer R. Levin, F M & Rudd E. Amer J Med 35 175 1963
14 Hijmans W Schuit H R. E. de Leeuw B & Mul ler H F Ned T Geneesk. 106 2097 1962
15 Hollister L. E & Hall, R. A Amer J Psychiat. 123 211 1966
16 Hallén, J Acta med. scand Suppl 462 1966
17 Mulhern, L. M Masi A T & Shulman, L. E. Lancet 2 508 1966
18 Seligmann M., Cannat, A. & Hamard M Ann N Y Acad. Sci. 124 816 1965
19 Weller T H & Coons A. H Proc Soc exp Biol. (N Y ) 86 789 1954

−6% This variation was in all patients insignificant compared with the day to day variation (Fig 3) On an average a small increase in pacing threshold was found during the day This increase was of a magnitude which could be expected from the day to day increase (Fig 4)

*Variations in pacing threshold related to meals sleeping and physical activity*

In ten patients several measurements were made just before and during the first hour after dinner A mean rise in pacing threshold of 2% was found one hour after the meal The greatest variations during the first hour were +16% and −4% In four of the patients no change in threshold values were seen

Examinations of pacing threshold during sleep were performed in ten patients A mean increase of 3% with extremes of +8 % and −3% was found after about 2 hours sleeping In two patients no changes were seen

In nine of ten patients a slight physical activity had no influence on pacing threshold In one patient a 4 decrease in threshold was seen

## DISCUSSION

In the present investigation as in previous reports (1 8 9) a mean increase in pacing threshold was found during the first days after insertion of

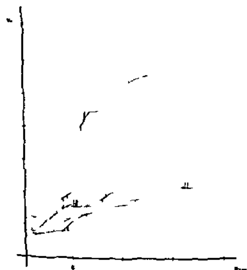


Fig 3 Day to day threshold variations in 9 patients (stippled lines) Variations during one day were examined in each patient These values are indicated as black columns on the stippled line The variations are seen to be small compared with the day to day variations

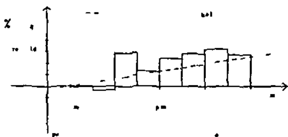


Fig 4 Mean threshold variations at two-hourly intervals during one day from 8 a m to midnight in 9 patients The first value in each patient = 100

the pacemaker electrode In some patients however an initial fall in pacing threshold was seen in the first few days followed by a subsequent rise Usually the threshold value was stabilized after 7 to 14 days A subsequent decrease in threshold to a level somewhat above the initial one as reported by others (1 8 9) was not generally seen

These day to day variations in threshold value may be of importance especially in the treatment of patients with myocardial infarction To obtain adequate and lowest possible stimulation daily threshold value determinations are necessary A too high pacemaker output may provoke ventricular arrythmias when fixed rate pacemakers are used or when the demand mechanism is failing which sometimes happ ns

No significant spontaneous variations were observed during the day A slight rise in the average pacing threshold during the day was observed but was within the range which could be expected due to the day to day rise following insertion of the electrode Variations up to 30 on either side of the mean have been reported to occur throughout the day (9) Such great variations in the threshold may possibly be due to unstable positioning of the electrode

The reported increase in pacing threshold after eating (7 9) and sleeping (7 8) and decrease after physical activity (7 8) cannot be confirmed by us The observed increases of 2 after meals and 3 during sleep are not significant After a slight physical activity no change in pacing threshold was observed

## REFERENCES

1 Albert, H M Glass B A Pittman, B & Robichaux, P Cardiac stimulation threshold chronic study Ann. N Y Acad Sci 111 889 1964

2 Davies J G & Sowton E Electrical threshold of the human heart Brit Heart J 28 231 1966
3 Furman S Parker B Escher D J & Solomon N Endocardial threshold of cardiac response as a function of electrode surface area J surg Res 8 161 1968
4 Grendahl H Sivertssen E Bay G & Bergan F Permanent cardiac pacing A follow up study of 88 patients Acta med scand 185 139 1969
5 Grendahl H & Sivertssen E Endocardial pacing in acute myocardial infarction Acta med scand 186 21 1969
6 Jàros G Marchand P Milner M & Obel I W P A long term study of threshold values and output characteristics of cardiac pacemakers Thorax 22 63 1967
7 Preston T A Fletcher R D Lucchesi B R & Judge R D Changes in myocardial threshold Physiologic and pharmacologic factors in patients with implanted pacemakers Amer Heart J 74 235 1967
8 Siddons H & Sowton E Cardiac pacemakers Thomas Springfield Ill 1967
9 Sowton E & Norman J Variation in cardiac stimulation threshold in patients with pacing electrodes Digest of the 7th international conference on medical and biological engineering Stockholm 1967

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# POSTTRANSFUSION MONONUCLEOSIS WITH HETEROPHIL ANTIBODIES

*Simultaneous Infection with Cytomegalovirus and EB Virus*

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*Abstract* A patient is presented with a virologically and serologically confirmed posttransfusion cytomegalovirus mononucleosis followed by the appearance of heterophil and EB virus antibodies. The patient received blood from one donor only having antibodies to both viruses. This fact is compatible with the supposition that the donor was a carrier of both cytomegalovirus and EB virus. It is suggested that there may be two kinds of posttransfusion mononucleosis, differing in causative agent and in clinical picture. One of these is caused by cytomegalovirus and is not attended by heterophil antibodies. The other is accompanied by a positive heterophil antibody test and usually by lymphadenopathy and is associated with EB virus or a closely related agent. The latter is *indistinguishable* from classical infectious mononucleosis or glandular fever. Both types were observed in the patient concerned

An infectious mononucleosis like disease with fever splenomegaly atypical lymphocytes and abnormal liver function tests may be observed after transfusion of fresh blood mostly in connection with surgical procedures. This posttransfusion syndrome differs usually from classical infectious mononucleosis or glandular fever by the absence of pharyngitis lymphadenopathy and heterophil antibodies. In 104 published cases however 11 patients had a more or less adequately documented positive heterophil antibody test. Rosenberg and Van Slyck (11) considered these Paul Bunnell positive cases to be instances of infectious mononucleosis

Kaariainen et al (7 8) provided evidence of cytomegalovirus infection in the Paul Bunnell negative posttransfusion syndrome which they called cytomegalovirus mononucleosis. Henle et al (6) have recently demonstrated the development of antibodies to EB virus in paired sera of patients with infectious mononucleosis. They used an indirect immunofluorescence test with a virus bearing cell line (EB 3) derived from a Burkitt lymphoma as antigen. The authors considered EB virus or a closely related agent as the aetiological agent of infectious mononucleosis. We now present a case of a serologically and virologically confirmed posttransfusion cytomegalovirus mononucleosis with evidence of a simultaneous EB virus infection

## MATERIAL AND METHODS

### *Virus isolation*

Urine mouth swabs and washed leucocytes were forwarded to the laboratory in a transport medium. Diploid human fibroblasts (GaBı strain) were inoculated and observed for six weeks. In the absence of a cytopathic effect, the culture was trypsinized and one blind passage was made. The second passage culture was observed for another week. Positive results were recognized in stained coverslip cultures by the typical cytopathic effect of cytomegalovirus

### *Complement fixation test*

A freeze-dried CF antigen was prepared with cytomegalovirus strain Davis in GaBı cells. The antigen was stabilized with 5% sorbitol. The dose of complement was 2 MHD. Fixation took place at 4–6 C overnight

### *Indirect immunofluorescence test for EB virus antibodies*

Sera were absorbed with dried rabbit kidney or liver powder and normal mouse brain suspension in egg yolk as described by Gispen and Brand Saathof (2) for rubella antibodies. The techniques of these authors for labelling and staining were closely followed. The sera were tested in twofold dilutions. The only modification was the use of 5% pepton rather than 10 normal guinea pig serum

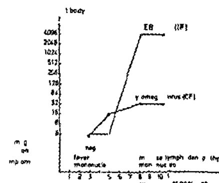


*Fig 1* Symptoms virus isolation and antibody response in a patient with posttransfusion mononucleosis with heterophil antibodies

in the diluent The EB 3 cells for IF procedures were cultivated for 3–4 days in Eagle MEM without arginine but with 10 newborn calf serum and 1 non-essential amino acids This procedure resulted in a fourfold rise of the number of fluorescent cells (5) EB 3 cells were spread on slides dried at room temperature and fixed in absolute methanol at −70 C for 30 min The slides were stored at −70 C The rabbit antihuman globulin conjugate was used in a constant dilution of 1 40 as lower dilutions gave a fine fluorescent stippling in some lymphoblasts with or without previous treatment with human serum The antibody titre was read at the highest serum dilution showing a definite though faint specific ng As positive control the serum of a 9 year old y with a Burkitt tumour (IF titre 1 2048) was chosen he sera of two 7 month-old children with minor illness erved as negative control

## RESULTS

The patient (H) described in this study is one of a series of four with posttransfusion mononucleosis in which we could demonstrate a cytomegalovirus infection (10) As this patient developed a positive Paul Bunnell reaction the level of EB virus antibodies was determined on different days in the course of her disease For reasons of comparison both cytomegalovirus and EB virus infection were also studied in a patient of the same age with posttransfusion mononucleosis without heterophil antibodies (W) and in a patient with classical infectious mononucleosis (K)

The blood donor was a 25 year-old healthy woman without a history of recent illness Physical examination performed one month after transfusion revealed no abnormalities The white cell differential count showed 59 % normal appearing lymphocytes and 1 % atypical lymphocytes The heterophil antibody test was negative Data on cytomegalo and EB virus serology are given below

## CASE REPORT

The patient (H) a 17 year-old girl received one unit of fresh blood during a splenectomy performed for idiopathic thrombocytopenic purpura Nine days later she developed fever which lasted 17 days There was no pharyngitis or lympadenopathy On the 16th postoperative day her white blood-cell count was 15 900 per mm with 37 mononuclear cells among which 49 were atypical lymphocytes Total serum bilirubin was < 1 mg/100 ml, serum alkaline phosphatase 10 0 Bessey units, serum-glutamic-oxaloacetic transaminase (SGOT) 46 units, serum glutamic pyruvic transaminase (SGPT) 11? units thymol turbidity 4 4 units (normal values serum alkaline phosphatase 0 8–2 3 Bessey units SGOT 5–40 units SGPT 5–35 units thymol turbidity < 5 units) The heterophil antibody test after guinea pig kidney absorption was negative The fever subsided gradually and the blood picture improved The patient was dismissed from the hospital 43 days after the operation but she returned two weeks later because of a general malaise At that time the white blood-cell count was 21 000 per mm with 83 mononuclear cells among which 39 were atypical lymphocytes The heterophil antibody titre was ≥ 51? On the 72nd postoperative day some tender slightly enlarged lymph nodes were palpable in the occipital region The temperature was normal Estimations of serum bilirubin serum alkaline phosphatase SGOT and SGPT gave normal results thymol turbidity was 15 8 units Symptoms disappeared gradually during the next few weeks

### *Virus isolation and serological response*

The results of the study are summarized in Fig and Table I In our patient H a significant increase of cytomegalovirus antibodies was found with the CF test at the end of the 5th postoperative week Cytomegalovirus was isolated from urine 58 days postoperatively when a considerable rise of titre was demonstrated for heterophil and EB virus antibodies

In serum derived from the donor one month after transfusion the heterophil antibody test was negative the cytomegalovirus antibody titre was 16 and the EB virus antibody titre 1024 Cytomegalovirus was not recovered from the donor urine or mouth swab

Paired sera of patient W with a Paul Bunnell negative posttransfusion mononucleosis yielded a cytomegalovirus antibody titre of 8 and 32 The EB virus antibody titre of both sera was 64 Cytomegalovirus was isolated from leucocytes on the 28th postoperative day and from urine on the

Table I *Virus isolation and serological response in patients with mononucleosis of different origin and in a healthy blood donor*

| Pat | Sex | Age | Clinical information | Day | Cytomegalovirus isolation | | Antibodies | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Urine | Mouth | Cytomegalovirus (CF) | EB virus (IF) | Heterophil |
| H | ♀ | 17 | Biphasic mononucleosis after splenectomy | 17 p o | | | | | — |
| | | | | 20 p o | Neg | Neg | — | — | |
| | | | | 34 p o | | | 16 | — | — |
| | | | | 58 p o | Pos | Neg | 32 | 4096 | ≥512 |
| | | | | 73 p o | | | 32 | 4096 | ≥512 |
| T | ♀ | 25 | Healthy blood donor of H | 1 mo after donation | Neg | Neg | 16 | 10 4 | — |
| W | ♂ | 17 | Mononucleosis after open heart surgery | 27 p o | | | — | 64 | — |
| | | | | 28 p o | Neg | Neg | | | |
| | | | | 63 p o | Pos | | 32 | 64 | — |
| K | ♂ | 23 | Infectious mononucleosis with lymphadenopathy | 4 | | | — | — | — |
| | | | | 14 | Neg | Neg | — | 1024 | ≥512 |

Cytomegalovirus isolation from leucocytes positive
p o = post operation
— = <8

63rd day The other patient (K) with classical mononucleosis had no antibodies against cytomegalovirus but did have a rising titre of EB virus antibodies together with the development of heterophil antibodies In this case no cytomegalovirus was isolated from mouth swab and urine on the 14th day of illness

## DISCUSSION

The findings in patient W with a Paul Bunnell negative cytomegalovirus mononucleosis show that EB virus antibody levels were not affected by cytomegalovirus infection This is in accordance with the results obtained by Henle and Henle (3) The presence of EB virus antibodies in this patient is not surprising as the incidence of these antibodies in a normal adult population is high (4 12) Patient K with classical infectious mononucleosis provides further evidence that EB virus or a closely related agent is associated with the aetiology of this disease The significant increase of antibodies to cytomegalovirus and EB virus in our patient with a Paul Bunnell positive posttransfusion mononucleosis indicates an infection with both cytomegalovirus and an EB like virus The former virus caused a typical posttransfusion cytomegalovirus mononucleosis the latter was associated with classical infectious mononucleosis with lymphadenopathy and heterophil antibodies

The presence of antibodies to both viruses in the donor indicates that exposure has occurred and is compatible with the supposition that she was a carrier of both viruses As posttransfusion mononucleosis has been observed only after transfusion of fresh blood the aetiological agent is probably transmitted with the blood Up till now cytomegalovirus has not been demonstrated in blood cells of healthy people Lang et al (9) succeeded in isolating the virus from leucocytes of patients with posttransfusion mononucleosis as we did in our patient W

A typical infectious mononucleosis with angina and lymphadenopathy has been transmitted by the experimental transfusion of 250 ml whole blood from a patient with glandular fever in the acute stage as reported by Wising (13) The incubation time in this case was 18 days It is possible that the longer incubation time in our patient was due to a smaller dose of virus Recent investigations by Diehl et al (1) demonstrated EB virus antigen in cultures of peripheral leucocytes from individuals with a history of infectious mononucleosis as long as 20 years ago

Posttransfusion mononucleosis may be differentiated into two entities according to the aetiological agent and the clinical picture One syndrome without heterophil antibodies and usually without lymphadenopathy is caused by cytomegalovirus The other with heterophil antibody re

sponse and usually with lymphadenopathy is brought about by EB virus or a closely related agent However the transmission of classical infectious mononucleosis by blood transfusion should be substantiated by the demonstration of EB virus in lymphocyte cultures of the donor or by the regular occurrence of EB antibody responses in patients

## REFERENCES


1 Diehl V., Henle G Henle W & Kohn, G Demonstration of a Herpes group virus in cultures of peripheral leucocytes from patients with infectious mononucleosis J Virol 2 663 1968
2 Gispen, R. & Brand Saathof B Assay of rubella antibodies by immunofluorescence complement fixation and neutralization. Arch ges Virusforsch 21 334 1967
3 Henle G & Henle W Immunofluorescence in cells derived from Burkitt s lymphoma J Bact. 91 1248 1966
4 — Immunofluorescence interference and complement fixation technics in the detection of the herpes type virus in Burkitt tumor cell lines Cancer Res. 27 2442, 1967
5 Henle W & Henle G Effect of arginine-deficient media on the Herpes-type virus associated with cultured Burkitt tumor cells. J Virol. 2 182, 1968
6 Henle G., Henle W & Diehl V Relation of Burkitt s tumor associated herpes-type virus to infectious mononucleosis Proc nat. Acad Sci (Wash) 59 94 1968
7 Kaariainen, L Klemola, E. & Paloheimo J Rise of cytomegalovirus antibodies in an infectious mononucleosis-like syndrome after transfusion. Brit. med. J 1 1270 1966
8 Kaariainen, L. Paloheimo J Klemola E Makela, T & Koivuniemi, A. Cytomegalovirus-mononucleosis Isolation of the virus and demonstration of subclinical infections after fresh blood transfusion in connection with open heart surgery Ann Med. exp Fenn. 44 297 1966
9 Lang, D J Scolnick, E. M & Willerson, J T Association of cytomegalovirus infection with the post perfusion syndrome New Engl. J Med 278 1147 1968
10 Langenhuysen, M M A. C & Kapsenberg, J G Cytomegalovirus-mononucleosis na bloedtransfusie Ned. T Geneesk. 112 2125 1968
11 Rosenberg, A. & Van Slyck, E. J A syndrome resembling infectious mononucleosis after splenectomy for idiopathic thrombocytopenic purpura. Ann. intern Med. 63 965 1965
12 Svedmyr A. & Demissie A. Age distribution of antibodies to Burkitt cells Acta path microbiol scand 73 653 1968
13 Wising, P J A study of infectious mononucleosis (Pfeiffer s disease) from the etiological point of view Acta med. scand. Suppl 133 1942.

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# MINERALOGICAL AND CLINICAL INVESTIGATION OF STONES FROM THE URINARY TRACT

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*Abstract* The investigation has been made on 106 stones from the urinary tract collected over a period of a little more than one year at Ullevål Hospital. The stones were examined mineralogically by the X ray diffraction method and the results are presented in tables. The patients from which the stones were taken have been classified according to the mineral composition of the stones and an attempt has been made to ascertain whether there are any clinical characteristics which can be correlated to the mineralogical classification

Too little is known about the causes of the formation of stones in the urinary tract to afford a basis for adequate treatment of the individual patient The clinician usually considers his task completed when the stone has been passed or removed by surgery However it is obvious that treatment which can prevent the formation of new stones will be possible only when it has been discovered what the physical basis of their origin might be A more precise diagnosis than merely urolithiasis might be possible by determining just which minerals are being precipitated and accumulated in the patient Clinical laboratories have been making chemical analyses of urinary calculi for quite some time but the results have proved of little use for diagnosis and therapy One of the reasons for this is that a chemical analysis as usually performed rarely allows of the classification of the individual patient into any special group The results of the chemical analysis usually give the chemical components which have been found without regard for what are the main or subsidiary components It must however be assumed that it is primarily the aggregation of the mineral which is the main component of the stone that characterizes the patient If the stone contains several minerals in approximately equal amounts a total chemical analysis is not very informative either In such cases it is the structural arrangement of the minerals which might tell us something about the causes of the aggregation

It seems practical and promising to classify the patients into groups according to the mineral or minerals of which their stones consist Even when there is a mixture of minerals in a stone a structural analysis shows that only a single mineral is crystallized and aggregated during any single period of the growth of the stone This again must indicate that the crystallization and aggregation of each mineral has its special cause It would therefore be of interest to determine whether patients with particular kinds of stones exhibit any common clinical characteristics which might suggest the pathological process which is responsible for the origin of the stones

The aim of the present study was to make a mineralogical examination of a chronologically and geographically limited material of stones of the urinary tract and to classify the patients into groups according to the mineralogical structure of the stones Furthermore an attempt has been made to determine whether there were any clinical characteristics which were specific for the individual groups

## METHOD

The method has been described earlier (10) Suffice it to mention here that the minerals in the stones were identified by the X ray diffraction technique using a 90 mm cylindrical Debye Scherrer camera and Mn filtered Fe irradiation. The stones were bisected with a circle saw The cut surface was examined under a dissecting microscope and described, and small samples were taken from apparently homogeneous areas with a dental drill When the stones were very small, the entire stone was crushed and examined as a single sample

Table I *The composition of 106 urinary calculi*

(*a*) 2 bladder stones (*b*) 3 bladder stones (*c*) 3 bladder stones (*d*) 1 prostate stone (*e*) 1 bladder stone (*f*) 1 prostate stone, (*g*) 2 bladder stones (*h*) 1 bladder stone The rest are from the kidney or ureter

| Chemical name | Formula | Mineral name | No of stones | of total |
|---|---|---|---|---|
| A *Mostly monomineral stones* | | | | |
| 1 Calcium oxalate monohydrate | $CaC_2O_4$ $H_2O$ | Whewellite | 12 (*a*) | 113 |
| 2 Calcium oxalate dihydrate | $CaC_2O_4$ $2H_2O$ | Weddelite | 18 (*g*) | 170 |
| 3 Calcium oxalate mono and dihydrate mixed | | | 16 | 151 |
| 4 Basic calcium phosphate apatite | $Ca_{10}(PO_4)_6(OH)_2$ | Hydroxyapatite | 16 (*b*) | 151 |
| 5 Magnesium ammonium phosphate hexahydrate (MAPH) | $MgNH_4PO_4$ $6H_2O$ | Struvite | 11 (*c*) | 104 |
| 6 Calcium hydrogen phosphate dihydrate (CHPD) | $CaHPO_4$ $2H_2O$ | Brushite | 3 (*d*) | 28 |
| 7 Uric acid | $C_5N_4H_4O_3$ | | 2 | 19 |
| 8 Cystine | $(SCH_2CH(NH_2)COOH)_2$ | | 2 | 19 |
| 9 Unidentified | | | 2 | 19 |
| B *Mixed stones* | | | | |
| 1 Calcium oxalate monohydrate + apatite | | | 5 | 47 |
| 2 Calcium oxalate dihydrate + apatite | | | 4 (*f*) | 38 |
| 3 Calcium oxalate mono and dihydrate + apatite | | | 2 | 19 |
| 4 Calcium oxalate monohydrate + MAPH | | | 1 ( ) | 09 |
| 5 Calcium oxalate dihydrate + MAPH | | | 1 | 09 |
| 6 Apatite + MAPH | | | 5 | 47 |
| 7 Apatite + CHPD | | | 1 | 09 |
| 8 Apatite + MAPH + calcium oxalate dihydrate | | | 1 (*h*) | 09 |
| 9 Apatite + calcium oxalate monohydrate + tricalcium phosphate | | | 1 | 09 |
| 10 Apatite + tricalcium phosphate | | | 1 | 09 |
| 11 Uric acid + calcium oxalate mono and dihydrate | | | 2 | 19 |

The Geology Museum of the University of Oslo helped us with the technical procedure with the X ray diffraction films We then identified the minerals by using reference films made with known compounds

## MATERIAL

The material was obtained from the Departments of Surgery and Urology at Ullevål Hospital Oslo on our request for surgically removed or spontaneously passed stones All the stones received at the laboratory from October 1964 to December 1965 a total of 106 were examined Among these there were 92 from the kidney or ureter 12 from the bladder and 2 from the prostate There were 86 removed surgically and 20 which had been passed spontaneously

## RESULTS

Table I shows the mineral composition of the stones examined

Table II shows the percentual occurrence of the different minerals in pure stones and in mixed stones

Table III is a comparison of the findings in per cent of *calcium oxalate* and *apatite* in some other urinary stone collections The table shows that several investigators have found calcium oxalate with about the same frequency in their materials However the frequency of monohydrate as compared to dihydrate varies somewhat The connection between the formation of the two hydrate forms is not clear Some authors believe that both forms are precipitated primarily (3 6 9) while others (5 7) believe that the dihydrate is precipitated primarily and can be recrystallized to the monohydrate An examination of Indian and Indonesian bladder stones (10) reveals a marked preponderance of monohydrate as compared to materials from the North Atlantic countries This may indicate that there are individual differences which determine which hydrated form is precipitated and that it may be of some hitherto unknown diagnostic significance to distinguish between the two forms

It is seen from Table III that apatite stones are usually mixed stones Table I shows what minerals were found in combination with apatite in our material What does not appear from the table is that apatite is not homogeneously mixed with

the other minerals but is found in isolated parts of the stones The so-called carbonate which is demonstrated in ordinary chemical analysis by the release of CO when the stones are dissolved in HCl was found in 33 % of the stones containing apatite It would seem that $CaCO_3$ has never been demonstrated mineralogically in urinary calculi Most authors believe hat CO takes the place of OH in the apatite crystals so that one might speak of a hydroxyl apatite and a carbonate apatite (3 6 9) Just as little is known about the cause of the formation of these two possible forms of apatite as about the origin of the two hydrated forms of calcium oxalate One hypothesis states that primarily precipitated hydroxyl apatite is secondarily transformed to carbonate apatite by taking up CO from alkaline urine (3) The proportions of hydroxyl apatite and carbonate apatite have been found to vary Lagergren (6) found CO in 100% of his apatite stones Herring (3) found 38% and in the present study we found 33 The diagnostic and therapeutic significance of distinguishing between these two alternative forms of apatite is not yet clear Table III shows that we have found pure apatite stones more frequ ntly than the other investigators although we are unable to provide any explanation of our finding

*Calcium hydrogen phosphate dihydrate* is seldom found in any material The same is true of *tricalcium phosphate* The three phosphates apatite and the two aforementioned will all be included under the designation phosphates in an ordinary chemical analysis There is however reason to believe that there are special conditions which lead to the precipitation and aggregation of each of these three minerals

*Magnesium ammonium phosphate hexahydrate* occurs relatively frequently in all materials and is aetiologically assumed to be related to infection with bacteria which break down urea

*Cystine stones* were found in 1–3 of all the materials (3 6 8) In these cases the aetiology is apparent as such stones are found only in cases of hereditary homozygous cystinuria

Table II *The percentual frequency of various minerals in pure stones and in mixed stones*

| | No of stones | of total |
|---|---|---|
| Calcium oxalate monohydrate pure stone | 12 | 11 3 |
| Calcium oxalate dihydrate pure stone | 18 | 17 0 |
| Calcium oxalate mono- and dihydrate pure stone | 16 | 15 1 |
| Calcium oxalate monohydrate in mixed stone | 11 | 10 4 |
| Calcium oxalate dihydrate in mixed stone | 10 | 9 4 |
| Magnesium ammonium phosphate hexahydrate pure stone | 11 | 10 4 |
| Magnesium ammonium phosphate hexahydrate in mixed stone | 8 | 7 6 |
| Apatite pure stone | 16 | 15 1 |
| Apatite in mixed stone | 20 | 18 9 |
| Calcium hydrogen phosphate dihydrate pure stone | 3 | 2 8 |
| Calcium hydrogen phosphate dihydrate in mixed stone | 1 | 0 9 |
| Uric acid pure stone | 2 | 1 9 |
| Ur c acid in mixed stone | 2 | 1 9 |
| Cystine pure stone | 2 | 1 9 |
| Tricalcium phosphate in mixed stone | 2 | 1 9 |
| Unidentified minerals pure stone | 2 | 1 9 |
| Unidentified minerals as trace in stones | 16 | 15 1 |

Table III *The percentual frequency of different minerals in various urinary stone collections*

| | *Lagergren* 1956 (6) | *Prien* 1949 (8) | Murphy and *Pyrah* 196? (7) | *Herring* 1962 (3) | Beeler et al 1964 (1) | Present in esti gation |
|---|---|---|---|---|---|---|
| Calcium oxalate monohydrate pure and mixed | 46 3 | 61 7 | 53 0 | 31 7 | 60 0 | 36 8 |
| Calcium oxalate d hydrate pure and m xed | 45 5 | 47 7 | 37 5 | 41 4 | 37 0 | 41 5 |
| Cal ium oxalate mono and dihydrate pur and mi ed | 50 5 | 67 0 | 60 0 | | | 59 4 |
| Apatite pure and mi ed | 74 0 | 51 8 | 59 5 | 6 8 | 43 0 | 34 0 |
| Apatite pure | 3 3 | 4 2 | 5 5 | | | 15 1 |

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of attacks was reported precisely (not only as "frequent attacks") the mean for all three groups is from 2.3–2.6 attacks before admission

VI *Information in case history as to specific diseases in relation to individual groups*

A search in the hospital records for other diseases gave the following findings in relation to the individual groups

*Patients with calcium oxalate stones (pure stones)*

1 tub pulm. 1 tub renis 3 inguinal hernias 2 urticaria, 1 ca renis, 1 aberrant renal vessel 3 familial occurrences of urinary calculi

*Patients with apatite stones (pure stones and apatite mixed with calcium oxalate)*

1 arthrosis coxae 1 transverse lesion with paraplegia, 1 infilt. pulm 1 ca. mammae 1 periurethral abscess 1 salpingitis 2 aberrant renal vessels 1 gonorrhoea, 3 syphilis.

*Patients with magnesium ammonium phosphate hexahydrate stones (pure and mixed stones)*

3 transverse lesion with paraplegia, 1 paralysed bladder (cause unknown) 1 tub pulm 1 aplasia renis 1 ca. renis 1 tub renis, 1 adenoma parathyreoideae 1 hypothyreosis 2 diabetes mellitus 1 podagra, 1 osteomyelitis 1 fractura colli femoris, 1 perinephritic abscess.

*Patients with calcium hydrogen phosphate dihydrate stones (pure and mixed stones)*

1 tub epididymidis 1 gonorrhoea, 1 aberrant renal vessel, 1 amp femoris (after accident)

*Patients with uric acid stones (pure and mixed stones)*

1 tub pulm., 1 polycythaemia, 1 adipositas

*Patients with cystine stones*

1 tub renis 1 psoriasis.

The survey shows information as to familial occurrence of urinary calculi in the calcium oxalate group In the same group there are three cases of inguinal hernia which are of interest.

In the apatite group three cases of syphilis in a total of 36 patients seems remarkably high

The calcium hydrogen phosphate dihydrate group is a small one of completely unknown aetiology It may be of interest that one of the patients who passed several prostate stones which proved to be of this composition suffered from a tubercular prostatitis

About 20% of all the patients had gastro-intestinal complaints but with no definite excess in any one group

VII *Clinico-chemical examinations*

The analyses usually carried out in serum of urinary calculi patients are creatinine urea Ca, P and alkaline phosphatase Table VII presents the pathological findings in the present material The relatively small number of pathological creatinine and urea values is surprising in view of the large number of previous attacks and information as to earlier pyuria

The table shows that there were some pathologically increased Ca values but there was no previous diagnosis of hyperparathyroidism for any of these patients Only one of the 106 patients had been operated for this disease previously This patient had magnesium ammonium phosphate stones and had normal Ca P and creatinine values

## ACKNOWLEDGEMENT

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## REFERENCES

1 Beeler M. F., Veith, D A., Morris, R. H. & Biskind G R., Techn. Bull. Reg. med Technol 34 57 1964
2. Falck Larsen, J & Philip J., Ugeskr Læg. 121 1993 1959
3 Herring, L. C., J Urol, 88 545 196–
4 Hughes, J., Coppridge W M., Roberts, L. C. & Mann, V L J Amer med. Ass. 17– 774 1960
5 Jensen, A. T Acta chir scand. 8 473 1941
6 Lagergren, C., Acta radiol. (Stockh.) Suppl. 133 19 6
7 Murphy B T & Pyrah, L. N Brit. J Urol. 34 129 1962.
8 Prien, E. L. J Urol. 61 821 1949
9 Prien, E. L. & Frondel, C J Urol. (Baltimore) 57 949 1947
10 Rokkones, T & Andersen, D A., Acta med. scand 183 431 1968

The adhesion in per cent was calculated from the formula

$$\frac{\text{initial count}-\text{final count}}{\text{initial count}} \times 100$$

When blood for platelet adhesion had been taken, 25 g glucose in a 50 aqueous solution was injected within 3 min for the intravenous glucose tolerance test (IVGTT) Blood glucose estimations from capillary blood samples before and 20 30 40 50 and 60 min after the injection were determined The half life of blood glucose was determined by graphic extrapolation of these values, and the result of the IVGTT was expressed as a $k$ value derived from the formula $0\ 693 \times 100/t$ representing the disappearance of blood glucose in per cent per min.

Correlations were tested by rank correlation according to Spearman, differences between groups by ranking according to Wilcoxon

The methodological error of determining platelet adhesion was obtained from duplicate estimations performed within half an hour and processed separately The error was 5 obtained from the formula $\sqrt{d^2/2n}$ where $d$ is the difference between paired estimations and $n$ the number of subjects.

## RESULTS

The results are presented in Table I The results of the platelet adhesion tests are shown in Fig 1 The mean value for platelet adhesion in the group with myocardial infarction was 44 6% ±2 4 This value did not differ significantly from the adhesive value of the control group which was 40 ±2 4 Nor was any difference seen when the younger patients arbitrarily taken to be those under 55

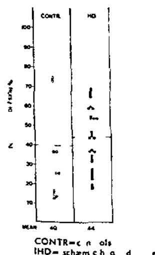



*Fig 1* Distribution of platelet adhesion values for 50 patients with ischaemic heart disease (●) and 50 controls (O)

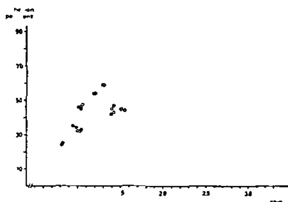


*Fig 2* Relationship between platelet adhesiveness and intravenous glucose tolerance ($k$ value) in 50 patients with ischaemic heart disease (●) and 50 controls (O)

years of age were compared with their controls No correlation was in fact found between platelet adhesion and age

Owing to the high rate of atherosclerotic disease associated with disorders of glucose metabolism (30) the $k$ values obtained from the IVGTT were compared to adhesion values in all subjects. The mean $k$ value for the group with ischaemic heart disease was 1 25±0 075 which is significantly lower ($P<0\ 02$) than the value of 1 48±0 085 for the healthy controls $k$ values were plotted against adhesion values (Fig 2) in both groups No correlations were obtained

In 15 arbitrarily chosen subjects both patients and controls platelet adhesiveness was determined before and 20 min after the 25 g intravenous glucose load A slight decrease in total platelet count was regularly obtained but adhesion values remained unaltered

The mean value for platelet adhesion in the *group of insulin treated diabetics* was 49 9% ± 3 13 This value is significantly higher ($P<0\ 05$) than that of the healthy control group but not when compared with the subjects with ischaemic heart disease

The mean triglyceride level of the group with ischaemic heart disease was significantly higher ($P<0\ 01$) 1 81±0 112 mmol/l compared with 1 33±0 106 mmol/l for the controls No correlations were found between triglyceride and platelet adhesion levels in either group The mean value for serum cholesterol was 259±5 93 mg/100 ml in the group with ischaemic heart disease and 253±8 67 mg 100 ml in the healthy controls

Table III *Previous investigations of platelet adhesion in patients with atherosclerosis*

| Author | Group studied | Method | Adhesion |
|---|---|---|---|
| Moolten et al 1949 (19) | Acute myocardial infarction | Moolten | Increased |
| Eisen et al 1951 (10) | Diverse forms of vascular disease including myocardial infarction and peripheral arteriosclerotic disease | Moolten | Normal in those with atherosclerotic vascular disease |
| McDonald & Edgill 1959 (16) | Angina acute coronary insufficiency and previous infarcts | Wright (modified) | Increased |
| Horlick 1961 (13) | Patients with 1 angina 2 infarct previous year 3 infarct prior to last year | Moolten | Increased in 1 and 2 but not 3 |
| Nestel 1961 (22) | Previous infarcts | Hutchison | Increased |
| Murphy & Mustard 1962 (21) | Patients with clinical complications of atherosclerosis angina infarcts carotid and basilar artery stenosis or occlusion intermittent claudication | Moolten | Increased |
| Moolten et al 1963 (20) | Arteriosclerotic heart disease angina infarcts | Moolten | Increased |
| Eastham 1964 (9) | Previous myocardial infarction on phenindione therapy | Easthams | Normal |
| Pfleiderer & Rucker 1964 (24) | Patients with peripheral arterial disease and patients with myocardial infarction treated with anticoagulants | Wright (Jurgens modification) | Increased |
| Kirby & Martin 1966 (15) | Peripheral and cerebrovascular arterial disease | Salzman | Normal |
| Baumgartner et al 1967 (1) | Patients with acute and sub-acute myocardial infarction and coronary heart disease | Hellem s | Increased |
| [illegible]rman et al 1967 (2) | Coronary artery disease | Wright (modified) | Increased |
| [illegible] & Stormorken [illegible]7 (25) | Previous infarcts | Hellem s PRP/ADP | Normal |

No correlations were obtained between platelet adhesion and serum cholesterol levels

## DISCUSSION

ADP induced platelet adhesiveness was found to be similar when estimated in a group of patients with ischaemic heart disease and normal controls No correlations between adhesion values and disorders of lipid or carbohydrate metabolism were found in these groups On the other hand platelet adhesion was found to be increased in a group of insulin treated diabetics ($P < 0.05$) No effect on platelet adhesion was found after an intravenous glucose load.

Several methods for estimating platelet adhesiveness have been developed (Table II) but few comparisons exist. Usually whole blood or platelet rich plasma (PRP) with added adenosine diphosphate (ADP) are used The latter is the substance R found by Hellem (11) to be present in erythrocytes causing platelets to adhere and which later was identified as ADP

When comparing the present findings with previous reports it is highly relevant to review comparisons made of the different methods for estimating platelet adhesion Horlick (13) when comparing Moolten and Vroman s method (18) with Wright s (31) could find no significant correlation between the two Hirsh et al (12) found a highly significant degree of correlation when comparing Wright s and Hellem s whole blood methods but this could not be confirmed by O Brien and Heywood (23) Sjogren et al (28) found a good correlation between Hellem s whole blood method and Salzman s method a weaker yet significant correlation between Wright s and Salzman s methods, whereas the correlation between Hellem s whole

blood and Wright s method fell short of statistical significance. These three whole blood methods were found not to be correlated to Hellem s PRP/ADP method

Direct comparisons of results in platelet adhesion studies must therefore be interpreted with care The varying methods may in part represent measurements of different parameters involved in the adhesive process

Most studies on patients with atherosclerotic disease employing whole blood methods have shown platelet adhesiveness to be increased as have two studies involving PRP only A survey of previous investigations of platelet adhesiveness in these patients is presented in Table III

On the other hand our findings are in agreement with those of Rozenberg and Stormorken (25) who also employed Hellem s PRP/ADP method using a final concentration of 0 05 μg ADP per ml PRP rather than 0 1 μg These authors investigated a group of patients with previous myocardial infarction and found similar degrees of platelet adhesiveness in these and a control group

Previous investigations on platelet adhesiveness in diabetes mellitus are presented in Table IV Most authors have used whole blood methods and found platelet adhesiveness to be increased in this condition Again our findings should preferably be compared with studies involving Hellem s PRP/ADP method Like Ödegaard et al (32) who used this method we found platelet adhesion to be increased yet to a less significant degree On the other hand the present study on patients with ischaemic heart disease and their controls fails to show any correlation between platelet adhesiveness and glucose tolerance

No effect on platelet adhesiveness was seen when results before and 20 min after an intravenous 25 g glucose load were compared Previous studies of this nature have been carried out with the help of whole blood methods and are therefore not satisfactory for comparison

As for the relationship between platelet adhesiveness and lipid metabolism our finding of a lack of correlation between adhesiveness and serum cholesterol and triglyceride levels are in agreement with those of Ödegaard et al (32) who employing the same adhesion method could find no correlation with cholesterol and total lipid values In this study cholesterol levels were similar in patients and controls while the triglyceride values were higher in the patient group This latter finding is in agreement with previous studies on patients with coronary heart disease in Stockholm (5 7) However the mean cholesterol in *those reports was also raised It is possible that* this difference at least partly might be attributed to the prescribed lowered intake of dietary fat

Table IV *Previous investigations of platelet adhesiveness in patients with diabetes mellitus*

| Author | Method | Adhesion |
|---|---|---|
| Moolten et al 1963 (20) | Moolten | Normal |
| Ödegaard et al 1964 (32) | 1 Hellem s whole blood | Decreased |
| | 2 Hellem s PRP/ADP | Increased |
| Bridges et al 1965 (3) | Wright (modified) | Increased |
| Kirby & Martin 1966 (15) | Salzman | Normal |
| Baumgartner et al 1967 (1) | Hellem s whole blood | Increased |
| Shaw et al 1967 (27) | 1 Eastham | Increased |
| | 2 Pegrum | Increased |

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## REFERENCES

1 Baumgartner H. R Cronquist, M Wobmann, P Streuli, F & Duckert F Die Messung der Thrombozytenadhäsivität. Erste Erfahrungen mit neueren Methoden. Schweiz. med Wschr 97 1674 1967
2 Besterman E. Myat, G & Travadi V Diurnal variation of platelet stickiness compared with effects produced by adrenaline Brit med J 1 597 1967
3 Bridges, J M Dalby A. M Millar J H D & Weaver J A. An effect of D-glucose on platelet stickiness. Lancet 1 75 1965
4 Carlson, L A Serum lipids in normal men Acta med scand 167 377 1960
5 — Serum lipid in men with myocardial infarction Acta med. scand. 167 399 1960
6 — Determination of serum triglycerides J Atheroscler Res. 3 334 1963
7 Carlson, L A. & Wahlberg, F Serum lipids, intravenous glucose tolerance and their interrelation studied in ischaemic cardiovascular disease Acta med scand 180 307 1966

8 Eastham R D Rapid adhesive platelet count in whole blood J Clin Path 17 45 1964
9 — Attempted reduction of adhesive platelets after cardiac infarction Lancet 2 541 1964
10 Eisen M E Tyson M C Michael S & Bauman F Adhesiveness of blood platelets in arteriosclerosis obliterans thromboangitis obliterans acute thrombophlebitis chronic venous insufficiency and arterio sclerotic heart disease Circulation 3 271 1951
11 Hellem A J The adhesiveness of human blood platelets in vitro Scand J clin. Lab Invest Suppl 51 1960
12 Hirsch J McBride J A & Wright H P Platelet adhesiveness a comparison of the rotating bulb and glass bead column methods Thrombos Diathes haemorrh (Stuttg) 16 100 1966
13 Horlick L Platelet adhesiveness in normal persons and subjects with atherosclerosis Amer J Cardiol 8 459 1961
14 Hutchison H E Stark J M & Chapman J A. Platelet serotonin and normal haemostasis. J clin Path 12 .65 1959
15 Kirby J C & Martin C L. Platelet adhesiveness and vascular disease Circulation Suppl 3 33 1966
16 McDonald L. & Edgill M Changes in coagulability of the blood during various phases of ischaemic heart disease Lancet 1 1115 1959
17 McDonald L Thrombosis in coronary heart disease Brit. Heart J 30 151 1968
18 Moolten S E. & Vroman L The adhesiveness of blood platelets in thromboembolism and hemorrhagic disorders I Measurement of platelet adhesiveness by the glasswool filter Amer J clin Path 19 701 1949
Moolten S E Vroman L & Vroman G M S Adhesiveness of blood platelets in thromboembolism and hemorrhagic disorders II Diagnostic and prog nostic significance of platelet adhesiveness Amer J clin Path 19 814 1949
20 Moolten S E Jennings P B & Solden A Dietary fat and platelet adhesiveness in arteriosclerosis and diabetes Amer J Cardiol 11 290 1963
21 Murphy E A & Mustard J F Coagulation tests and platelet economy in atherosclerotic and control subjects Circulation 25 114 1962
22 Nestel P J A note on platelet adhesiveness in ischeamic heart disease J clin Path 14 150 1961
23 O Brien J R & Heywood, J B A comparison of platelet stickiness tests during an Atromid S trial Thrombos. Diathes haemorrh (Stuttg.) 16 768 1966
24 Pfleiderer T & Rucker G Über Kaliumgehalt und Adhäsibität der Thrombocyten von gesunden Men schen und Patienten mit obliterierender Gefass Skle rose Klin Wschr 42 82, 1964
25 Rozenberg, M C & Stormorken M Comparison of glass adhesiveness and rate of aggregation of blood platelets. Scand J. clin Lab Invest 19 82 1967
26 Salzman E. Measurement of platelet adhesiveness J Lab clin Med 62 724 1963
27 Shaw S Pegrum G D Wolff S & Ashton W L. Platelet adhesiveness in diabetes mellitus J clin Path 20 845 1967
28 Sjogren, A., Bottiger L. E. & Biorck G Platelet adhesion a comparison of four methods Acta med. scand 185 127 1969
29 Stormorken H Lund Ruse A. & Rørvik T O Platelet adhesiveness to glassbeads methodological investigation using automatic particle counting. Scand. J clin Lab Invest Suppl 84 183 1965
30 Wahlberg F Intravenous glucose tolerance in myocardial infarction angina pectoris and intermittent claudication Acta med scand Suppl 453 1966
31 Wright H P The adhesiveness of blood platelets in normal subjects with varying concentrations of anticoagulants. J Path Bact 53 255 1941
32 Ödegaard A E. Skålhegg, B A. & Hellem, A J Increased activity of Anti Willebrand Factor" in diabetic plasma Thrombos Diathes haemorrh (Stuttg.) 11 27 1964

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# A TWO YEAR CIRCULATORY FOLLOW UP OF PHYSICAL TRAINING AFTER MYOCARDIAL INFARCTION

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*Abstract* Circulatory effects of physical training after myocardial infarction have been evaluated in six middle aged men. The schedule consisted of studies before training, after a two month s programmed training, and after an additional two years spontaneous physical a tivity After two months three patients stopped the excess activity while three continued it at different levels.

Exercise tolerance measured by ergometry increased in all subjects after two months. The symptoms occurred at identical heart rates but external work loads evoking these heart rates were higher After additional two-year activity both the loads and heart rates tolerated were hi her In the subgroup who stopped the activity exer cise tolerance was largely at the pretraining level.

A decrease in tension time index and no change in heart volume explained the change at two months. The same parameters could be invoked to explain the deteriora tion of the inactive group after two years. After continued excess activity tension time indices during exercise were higher in two patients than the level at which symptoms occurred before training. Changes in heart volume were small after two years.

It is concluded that short-term and long term training responses differ in the mode by which they increase exer cise tolerance After two months training hemodynamic changes decreasing oxygen demand of the heart are suf ficient to explain the progress, whereas after two years activity additional so far unverified, factors are invol ed

As one of the procedures in the secondary preven tion of coronary heart disease physical training is gaining increasing acceptance and is currently widely practised (1 4 11 12 14 15 16 20 21 22 23 24) A unanimous experience has been a clearcut decrease in morbidity as reflected in de creased number and severity of anginal attacks and in increased exercise tolerance Contrary to this multicenter evidence studies made in the ef fect to evaluate the hemodynamic factors respon sible for this change are few (8 30) and attempts to visualize changes in coronary arteries are sporadic

In this report circulatory data are presented of a pilot study (8) extended to cover a period of 26 months of physical activity after myocardial infarction

## MATERIAL

Six male patients, aged 37–55 in December 1966, to whom it was explained that the study consisted of re peated cardia catheterizations, were selected on the basis of their willingness and possibilities to participate in the trial All were in sinus rhythm and had had a myocardial infarction from 2–4 months before the beginn ng of the training. In two patients the infarction came out of the blue" while four of the patients had had angina pectoris for varying legnths of time After the infarction one of the patients was free of symptoms on ord nary daily phys cal activity

In four of the patients the heart volume exceeded 500 ml m² Hematological studies excluded anemia, and respiratory functions were normal

## METHODS

*Training p ogram*

The programmed training, described n detail earlier (8) consisted of work on an electrically braked ergometer (Elema-Schonander) three times a week for a period of approximately two months. The training session was composed of a low warm-up" load, a load increasing the heart rate o er 100/min, and two successi ely in creased loads up to chest pain and or dyspnea During the weeks of training these loads were gradually increased according to each subject s tolerance

After this programmed training was finished the pa tients were encouraged to continue daily excess phys cal acti ity This succeeded in three patients, cases 2, 3 and 6 the activity being the highest in case 6. This man jogged or ran 8–10 km daily and 15 km during the week-ends. Case 2 walked daily 6 km at a speed up to dyspnea, and in case 3 the daily walking distance was about 5 km.

Table I *Effect of physical training on exercise tolerance*

| Case no | State | Load (kpm/min) | Heart rate (beats/min) | Symptoms |
|---|---|---|---|---|
| 1 | BT | 600 | 145 | Dysp VEB |
| | AT 2 mo | 600 | 118 | VEB |
| | 2 y | 600 | 147 | AP dysp VEB |
| 2 | BT | 600 | 142 | Dysp |
| | AT 2 mo | 600 | 132 | — |
| | AT 2 mo | 750 | 156 | — |
| | AT 2 y | 600 | 1?2 | — |
| | AT 2 y | 900 | 176 | — |
| 3 | BT | 400 | 140 | AP |
| | AT 2 mo | 400 | 123 | — |
| | AT 2 mo | 00 | 148 | AP |
| | AT 2 y | 450 | 121 | — |
| | AT 2 y | 900 | 167 | AP |
| 4 | BT | 450 | 135 | — |
| | BT | 600 | 153 | Dysp |
| | AT 2 mo | 450 | 127 | — |
| | AT 2 mo | 600 | 142 | — |
| | 2 y | 400 | 120 | AP dysp |
| 5 | BT | 600 | 136 | AP |
| | AT 2 mo | 600 | 121 | — |
| | AT 2 mo | 650 | 127 | — |
| | 2 y | 650 | 145 | Dysp |
| 6 | BT | 400 | 150 | AP |
| | AT 2 mo | 400 | 128 | — |
| | AT 2 mo | 600 | 150 | AP |
| | AT 2 y | 500 | 140 | — |
| | AT 2 y | 700 | 157 | — |
| | AT 2 y | 900 | 168 | Leg exhaustion |

BT = before training AT = after training VEB = ventricular ectopic beats AP = angina pectoris

Cases 4 and 5 stopped the excess activity after the programmed period due to disinterest In case 1 palpitations and dizziness developed and he could not continue excess activity after having been fairly active for a year after the initial training.

*Exercise tolerance test*

The subjects pedalled the electrically braked ergometer in sitting position starting from low loads up to a load causing angina pectoris or dyspnea to the degree that they would have stopped if they had been free to do so Every load lasted for 4 min and it was increased without an intervening pause The ECG was continuously monitored on a 4-channel Airmec oscilloscope and recorded every minute by a six-channel Elema recorder The registered CH loads were V . The exercise tolerance was evaluated both as the load in kpm min which the patients were able to pedal for 4 min and during which the symptoms appeared and as the heart rate at the moment of distress. The symptoms were always preceded by S-T segment depressions of ischemic type (3)

*Circulatory studies*

The heart volume was measured from chest X rays taken in erect position and without ECG timing (18)

Hemodynamic measurements were made at rest and during supine exercise at loads selected by previous testing The resting base line was taken with the subjects feet on the ergometer pedals (5) Right sided cardiac catheterization and direct recording of brachial arterial pressure were performed before and after the programmed training These results have been reported earlier (8). The patients could not be persuaded to undergo a third complete evaluation after the period of 6 months and during the last testing only heart rate oxygen consumption and brachial arterial pressure were recorded by the equipment and methods earlier described (8) Left ventricular ejection time was rate-corrected according to a formula calculated from normal subjects (7) and labelled left ventricular ejection time index (LVETI) The tension-time index (TTI) of Sarnoff et al ( 8) was calculated from the intra arterial pressure tracings run intermittently at a speed of 100 mm/sec

Only the parameters measured in the last examination are reported for the two earlier study sessions The significance of the difference of the means was calculated by Student s *t* test

## RESULTS

*Exercise tolerance*

After two months training all the patients had better tolerance than before training This was mainly in the form of an increased external load tolerated but the symptoms appeared approximately at the same heart rates as in the beginning (Table I) After a further two years the subjects could be divided into two subgroups (A) cases 1 4 and 5 who had stopped the activity and (B) cases 2 3 and 6 who continued the activity The subgroup A was largely at the pretraining level while in group B all the subjects had better exercise tolerance than after two months training (Table I)

*Circulatory data*

The mean heart volume on leaving the hospital after the infarction was $922 \pm 173$ ml (S D ) During the period up to the beginning of the training the mean volume increased to $989 \pm 219$ ml ($P < 0.01$) Individually this increase occurred in five of the patients a decrease occurring in one (Fig 1) The programmed training resulted in a decrease in four and increase in two patients The mean volume of $935 \pm 146$ ml was not significantly different from the pretraining value After continued excess activity the heart volume decreased in all subjects in question After the cor

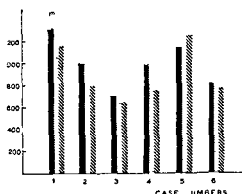



*Fig 1* Individual responses of heart volume (HV) to physical activity The different columns from left to right at discharge from hospital at the beginning of training after 2 months training and after 2 years activity ( ) or inactivity

responding period of no excess activity the vol ume increased in cases 1 and 5 and decreased in case 4 (Fig 1)

The circulatory data were not significantly changed either after two months or two years training when recorded with the patients resting supine (Table II) After two months training significant changes became apparent during exer cise in the heart rate ventilation oxygen uptake left ventricular ejection time index and tension time index (Table II) After an additional two years activity the heart rate was lower during exercise in every subject than after two months training (subgroup B) whereas after a correspond ing period of inactivity the heart rate was higher in every subject than after the programmed train ing (subgroup A Table II) In this group the

Table II *Group averages of data before training and after 2 months and 2 years training*

| | | HR (beats/ m n) | $V_E$ (l/min) | $V_O$ (ml min) | $P_{BA}$ S | $P_{BA}$ D | $P_{BA}$ M | LVETI (msec) | TTI (mm Hg/ min) |
|---|---|---|---|---|---|---|---|---|---|
| *Total group* | | | | | | | | | |
| Rest | BT | 76 | 7 6 | ⌞80 | 136 | 77 | 97 | 381 | 2519 |
| (*n*=6) | | ±11 | ±1 7 | ±35 | ±26 | ±15 | ±15 | ±16 | ±504 |
| | AT 2 mo | 78 | 8 9 | 291 | 137 | 77 | 97 | 397 | ⌞941 |
| | | ±10 | ±2.6 | ± 42 | ±21 | ±9 | ±12 | ±14 | ±670 |
| *P* | | n s | n s | n s | n s | n s | n s | n s | n s |
| Exercise | | | | | | | | | |
| Load 1 | BT | 1⌞9 | ⌞6 7 | 1085 | 182 | 96 | 126 | 417 | 5316 |
| (*n*=6) | | ±14 | ±4 6 | ±178 | ±30 | ±12 | ±17 | ±16 | ±909 |
| | AT 2 mo | 112 | 24 4 | 1007 | 179 | 97 | 128 | 402 | 4812 |
| | | ±14 | ±4 2 | ±179 | ±26 | ±18 | ±21 | ±18 | ±1061 |
| *P* | | 0 01 | 0 05 | 0 01 | n s. | n s | n s | 0 02 | 0 01 |
| Load 2 | BT | 133 | 39 4 | 1557 | 197 | 103 | 132 | 413 | 5168 |
| (*n*=4) | | ±10 | ±6 1 | ±299 | ±27 | ±⌞0 | ±25 | ±18 | ±867 |
| | AT 2 mo | 122 | 38 1 | 1527 | 199 | 95 | 129 | 393 | 4382 |
| | | ±11 | ±4 9 | ±229 | ±41 | ±16 | ±27 | ±19 | ±703 |
| *P* | | 0 02 | n s | n s | n s | n s | n s | n s | 0 05 |
| *S bg o p A* | | | | | | | | | |
| Rest | AT 2 mo | 72 | 11 1 | 318 | 141 | 81 | 94 | 388 | 2912 |
| (*n*=3) | 2 y inact | 79 | 10 9 | 306 | 149 | 80 | 103 | 365 | 2909 |
| Exercise | | | | | | | | | |
| Load 1 | AT 2 mo | 100 | 25 3 | 1046 | 182 | 98 | 131 | 393 | 4603 |
| | 2 y inact | 115 | 24 2 | 1023 | 177 | 87 | 119 | 377 | 4 02 |
| Load 2 | AT 2 mo | 1 0 | 36 6 | 1552 | 208 | 98 | 133 | 393 | 4444 |
| | 2 y inact | 138 | 34 3 | 1310 | 196 | 93 | 129 | 384 | 5143 |
| *Subg oup B* | | | | | | | | | |
| Rest | AT 2 mo | 84 | 6 7 | 256 | 133 | 79 | 100 | 406 | 2771 |
| (*n*=3) | AT 2 y | 90 | 10 1 | 288 | 135 | 75 | 101 | 394 | 3081 |
| Exerc se | | | | | | | | | |
| Load 1 | AT 2 mo | 127 | 25 8 | 1098 | 177 | 97 | 1 6 | 411 | 4778 |
| | AT 2 y | 120 | 22 5 | 1076 | 173 | 89 | 123 | 424 | 5051 |

HR = heart rate $V_E$ = ventilation $V_O$ oxygen uptake $P_{BA}$ = brach al arterial pressur S = systol c D = diastolic M = mean LVETI = left ventricular ejection time index TTI = tens on time index BT = before train ng AT = after tra ning.

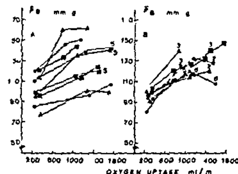



*Fig 2* Brachial arterial mean pressures at rest and during exercise in relation to oxygen uptake ● = before training Δ = after 2 months training and ◻ = after 2 years inactivity (A) or 2 years activity (B) Individual case numbers are also shown

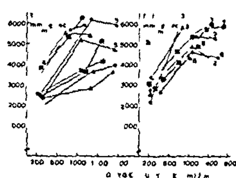


*Fig 4* Tension time indices (TTI) at rest and during exercise in relation to oxygen uptake Symbols as in Fig 2

higher level of exercise labelled load 2 was clearly too much for the subjects resulting in angina and clumsy pedalling at a rate below accuracy Expired air could only be collected during 2–4 min of exertion and the lower mean values of oxygen consumption at this load (Table II) was presumably a reflection of a non steady state

No change in brachial arterial pressure was seen after two months training Neither could any change be detected after the additional two years but smaller individual variations occurred in subgroup B than A (Fig 2) A directionally opposite change was observed in the left ventricular ejection time index which was significantly shorter after two months training ($P < 0.02$) but longer in every subject during exercise after an additional two years activity (Table II Fig 3)

The tension time index was reduced after two months training (Table II) With continued activity it remained below the pretraining value in case 2 in spite of additional higher loads at two years (Fig 4) while in cases 3 and 6 it was higher at identical loads than after two months training the mean value of the whole subgroup B being higher than after the programmed period (Table II)

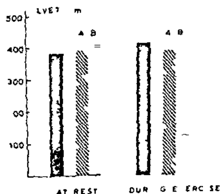



*Fig 3* Mean left ventricular ejection time indices (LVETI) at rest and during exercise The columns from left to right before training after 2 months training after 2 years inactivity (A) and continuous activity (B)

## DISCUSSION

In evaluating the results after two months physical training some suspicions were expressed about the observed changes being partly caused by the training and probably partly by the prolonged convalescence (8) The significant increase in the heart volume observed during the period of dismissal from hospital to the beginning of the training reflects in all probability the disappearance of the hypokinemia caused by bed rest during the acute phase of the illness (27) After this no significant change was observed during the period of programmed training After an additional two years of activity the heart volume slightly decreased in all the relevant patients (Fig 1) This response to training is far from typical as portrayed in numerous articles and recently reviewed (9) The normal response has uniformly been an increase but the series has been composed of normal subjects of an age considerably lower than the present patients Recently training responses in middle aged men were reported but data on heart volume were omitted (13) Earlier attempts include small groups corresponding in age to our series and no increase in heart volume was observed (17) Training has not been found to increase the heart volume in old persons (?) It thus appears that the "abnormal response of the

heart volume seen in the present study is related to the age of the patients and not necessarily to the presence of coronary heart disease The role of subclinical coronary artery disease in any series of corresponding age cannot of course be excluded

The decrease in the heart rate during exercise after two months training continued in the active group during the additional two years observation time and represents a true training response from the very beginning being analogous to the training effect in series of healthy subjects verified by numerous studies This is further substantiated by the return of the heart rate to the pretraining level in the group who discontinued the excess activity being an example of the functional nature of the change (6)

With regard to arterial pressure the response to both short and long term training was commensurate with findings in normal series showing no change in directly measured arterial pressures either at rest or during exercise (9) The shorter left ventricular ejection during exercise found after two months training gives further evidence for an increased force and rate of cardiac ejection when coupled with the data earlier reported (8) showing larger stroke volumes and better ventricular functions after training Exactly the same observation was recently made in studying seven months training response in middle aged men (13) After the additional two years of activity in the present study left ventricular ejection was not different at rest but longer in every subject during exercise Owing to the lack of relevant supplementary data various mechanisms can only be speculated upon This type of change could be due to either decreased sympathetic drive decreased intrinsic contractility or larger ejection volume in combination with unchanged or increased contractility Considering the overall picture of decreased heart rates and largely unchanged heart volumes a decreased sympathetic drive coupled with larger stroke volumes due to better contractile state is the likely solution The trend to shorter left ventricular ejection in the group who stopped the training could be explained analogously by invoking smaller stroke volumes and enhanced sympathetic drive concordant with the increased heart rates

The rate pressure product has experimentally been shown to be a reliable indicator of myocardial oxygen consumption (19 26 28) This parameter has clinically been successfully employed either by using muscular exercise (25) or atrial pacing (29) to depict the pain threshold in patients with angina pectoris as well as to elucidate the effect of nitroglycerin thereupon (10) After two months training the reduced tension time indices coupled with largely unchanged heart volumes are sufficient to explain the better exercise tolerance found concomitantly After the two-year period of inactivity the same factors can be held responsible for the deterioration to the pretraining level After 26 months excess physical activity the tension time indices in two subjects were higher than the level at which pain was produced before training and one of these patients could be pushed to exhaustion on the ergometer (Table I) without any chest pain An explanation of this might be the smaller heart volume reducing the oxygen requirement via decrease of tension according to LaPlace s law or a more synchronized contraction instead of the asynchrony known to occur in the ventricle damaged by infarction An attractive but unproved alternative is enhanced blood supply through collaterals

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## REFERENCES

1 Barry A J Daly J W Pruett E D R Steinmetz, J R Birkhead N C & Rodahl K Effects of physical training in patients who have had myocardial infarction Amer J Cardiol 17 1 1966
2 Benestad A M Trainability of old men Acta med scand. 178 321 1965
3 Blackburn, H Keys A Simonson E Rautharju P & Punsar S The electrocardiogram in population studies Circulation 1 1160 1960
4 Brunner D The influence of physical activity on incidence and prognosis of ischemic heart disease In Prevention of ischemic heart disease (ed W Raab) p 436 Thomas, Springfield Ill 1966
5 Frick M H & Somer T Base line effects on response of stroke volume to leg exercise in the supine position J Appl Physiol 19 639 1964
6 Frick M H Elovainio R O & Somer T The mechanism of bradycardia evoked by physical training Cardiologia 51 46 1967
7 Frick M H Somer T & Elovainio R. O Effect of physical training on left ventricular systole Cardiologia 51 33 1967

8 Frick, M H. & Kaula, M Hemodynamic consequences of physical training after myocardial infarction. Circulation 37 192, 1968

9 Frick, M H. Coronary implications of hemodynamic changes caused by physical training. Amer J Cardiol. 22. 417 1968

10 Frick, M H., Balcon, R., Cross D & Sowton, E. Hemodynamic effects of nitroglycerin in patients with angina pectoris studied by an atrial pacing method. Circulation 37 160 1968

11 Gottheiner V Long range strenuous sports training for cardiac reconditioning and rehabilitation. Amer J Cardiol. 22. 426 1968

12. Hakkila, J & Rinne H. Rehabilitation of patients with myocardial infarction and patients after heart surgery Rep Inst. Occup Health (Helsinki) no 30 1965

13 Hanson, J S., Tabakin, B S., Levy A. M & Nedde W Long-term physical training and cardiovascular dynamics in middle aged men. Circulation 38 783 1968

14 Hellerstein, H. K., Hirsch, E. Z. Cumler W., Allen, L., Polster S & Zucker N Reconditioning of the coronary patient. Preliminary report. In Coronary heart disease (eds. W Likoff & J H Moyer) p 448 Grune & Stratton, New York 1963

15 Hellerstein, H K. Rehabilitation of patients with heart disease Postgrad. med. 15 265 1964

16 Hellerstein, H. K., Hornsten, T R., Goldbarg, A., Burlando A. G., Friedman, E. H., Hirsch E Z. & Marik, S Influence of active conditioning upon subjects with coronary artery disease Canad. med. Ass. J 96 758 1967

17 Holmgren, A., Mossfeldt, F., Sjostrand T & Strom, G Effect of training on work capacity total hemoglobin, blood volume, heart volume and pulse rate in recumbent and upright positions. Acta physiol. scand. 0 72, 1960

18 Jonsell, S Method for the determination of the heart size by teleroentgenography (heart volume index) Acta radiol. (Stockh.) 20 325 1939

19 Katz, L. N The factors involved in the regulation of hearts performance In. Circulation, Proc Harvey tercentenary congr (ed J McMichael) p 70 Blackwell, Oxford 19 8

20 Kaufman, J M & Anslow R. D Treatment of refractory angina pectoris with nitroglycerin and graded exercise J Amer med. Ass. 196 151 1966

21 Kellermann, J J., Levy M., Feldman, S & Kariv L. Rehabilitation of coronary patients. J chron. Dis. 20 815 1967

22. Kellermann, J J., Modan, B Levy M Feldman, S & Kariv I. Return to work after myocardial infarction. Comparative study of rehabilitated and nonrehabilitated patients. Geriatrics 23 151 1968

23 McAlpin, R. B & Kattus, A. A. Adaptation to exercise in angina pectoris. Circulation 33 183 1966.

24 Naughton, J & Balke B Effect of physical training on work capacity in post myocardial infarction patients. J Sport Med. 4 185 1964

25 Robinson, B F. Relation of heart r te and systolic blood presure to the onset of pain in angina pectoris Circulation 35 1073 1967

26 Rolett, E. L., Yurchak, P M., Hood, W B Jr & Gorlin, R. Pressure volume correlates of left ventricular oxygen consumption in the hypervolemic dog Circulat. Res. 17 499 1965

27 Saltin B., Blomqvist, G., Mitchell, J H., Johnson, R. L Jr Wildenthal, K. & Chapman, C. B Response to exercise after bed rest and after training. Circulation Suppl. 7 1968

28 Sarnoff S J., Braunwald, E. Welch G H. Jr Case, R. B., Stainsby W N & Macruz, R. Hemodynamic determinants of oxygen consumption of the heart with special reference to tension-time index. Amer. J Physiol 192 148 1958

29 Sowton G E., Balcon R., Cross, D & Frick, M H Measurement of the angina threshold using atrial pacing. Cardiovasc. Res 1 301 1967

30 Varnauskas, E., Bergman H., Houk, P & Björntorp P Haemodynamic effects of physical training in coronary patients. Lancet 2 8 1966

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# ENZYMATIC PATTERN OF LIVER INJURY IN DUPUYTREN S CONTRACTURE

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*Abstract* In two groups of patients with Dupuytren s contracture the first of which consisted of patients operated upon, and the second of patients hospitalized for various reasons in whom the contracture was discovered by chance indicator and excretory serum enzymes have been determined A statistically highly significant increase of serum leucine amino peptidase and γ-glutamyl transpeptidase was found There was a simultaneous increase of serum alkaline phosphatase as well, but this rise was not statistically significant Accompanying diseases were represented chiefly by ethylism and diseases of the liver or gastrointestinal tract.

As regards the etiology of Dupuytren s contracture (D C) references have been made to gout, rheumatism arthrosis deformans diseases of thyroid gland and parathyroid diabetes mellitus tuberculosis specific and non specific infection hepatic disturbances diseases of vegetative and peripheric nerves metabolic disturbances and traumas According to different authors (7 15 16 17 18) D C is a symptom of liver cirrhosis especially the alcoholic type Nevertheless the reports differ in the percentage of occurrence of D C in cirrhotic patients to a great extent

It was the aim of our investigations to find possible signs of hepatic lesion in patients with D C Therefore the enzymes—excretory as well as of indicator type—applying to liver parenchyma were used due to the fact that common liver tests (thymol turbidity test Kunkel s test bilirubinemia cholesterol in serum and paper electrophoresis) that we carried out at the same time did not present any typical or convincing results

## MATERIAL AND METHODS

The patients originate either directly from the town of Brno or from near surroundings Most patients were not manual workers. There were only two miners one brick layer three bus drivers one pilot and one gymnast

There were two groups of patients.

The first group consisted of 90 patients who underwent the operation for D C in the years 1952–1954 in plastic surgery and who were checked in the years 1966–1967 in the outpatient department of the clinic It was a random systematic choice of patients from the total of 750

Group I between 80–35 years the average age being 59¹/ years In all these cases D C was advanced and relapses occurred in most of them

The second group cons sted of 35 patients hospitalized in the years 1966–1967 for different reasons. In these patients—it was a simple knot of the palmar aponeurosis—D C was found at random.

Group II between 90–42 years the average age being 65 1 years There was a clear predominance of men.

There were only six women (6 6%) in the first group and eight women in the second (22.8 %)

We carried out the following laboratory tests determination of enzyme activity of transaminase glutamate oxalate (GOT) transaminase glutamate pyruvate (GPT) leucine amino peptidase (LAP) γ glutamyl tran.pepudase (GGTP) alkaline phosphatase (AP) and lactic dehydrogenase (LDH)

Transaminase of glutamate-oxalate and glutamate pyruvate were determined by the method of Reitman and Frankel (9) modified by Sevela (13) Values up to 1 5 μmol GPT and 1 3 μmol GOT are considered normal Serum leucine amino peptidase (substrate L leucylbeta naphthylamide) was assessed according to Goldbarg s and Rutenburg s method (3 1 ) The activity of enzyme was determined as re ards other enzymes, in micromoles of enzymatically split substrate after conversion to 1 ml of serum and 1 hour incubation at 37 C The values exceeding 3 5 μmol are considered pathologic γ-glutamyl transpeptidase was determined by the method of Kulhánek and Dimov (5) The values exceeding 1 5 μmol are considered patholog c Alkaline phosphatase was deter m ned according to Bessey et al (1) Values exceed ng 3.5 μmol are considered pathologic Lactic dehydrogenase was determined by the method of Še ela and To areL (14) Values up to 10 μmol a e normal In statistical evaluation Student s t test was used

Table I *Changes of excretory en ymes in both groups of patients with D C*

| Enzyme | No of determinations Group I | Group II | No of raised values Group I | Group II | Total case | Percentage | Statistical significance |
|---|---|---|---|---|---|---|---|
| LAP | 90 | 34 | 42 | 19 | 61 | 49 1 | $p<0\ 1$ |
| GGTP | 48 | 23 | 11 | 9 | 20 | 28 1 | $p<0\ 1$ |
| AP | 72 | 22 | 13 | 6 | 19 | 20 2 | $p>5$ |

## RESULTS

The activity of the indicator enzymes GOT GPT and LDH in serum was examined in all 125 patients but there were rare and slight changes only In 11 patients i e 8 8% the GPT was increased An increase of GOT was found in two cases only In 13 patients i e in 10 4% an increase of LDH was found All the cases mentioned—with three exceptions—belonged to the second group

Changes of the excretory enzymes are shown in Table I LAP was examined in all patients from the first group and in all but one in the second The elevation took place in 49 1% together and this increase was statistically very significant P < 0 1 %) The same statistically very significant crease was ascertained in serum with GGTP was determined in 71 patients—in 48 from the first group and in 23 from the second There was a total elevation in 28 1% The elevation with AP in serum which was determined in 94 patients was not statistically significant ($P>5$)

In the next part of our study we were concerned with associated diseases of the patients

Apart from the family occurrence of D C which manifested itself especially in the first group where it reached even 26 6% diseases directly or indirectly affecting the liver parenchyma or biliary ducts prevailed There was a clear prevalence of ethylism (roughly in every third case) followed by chronic gastroduodenal ulcer and chronic cholecystitis There was a different occurrence of cirrhoses and of diabetes mellitus in the two groups In the group of hospitalized patients tolbutamide tests were carried out in 12 patients (Patients with manifest diabetes and coronary heart disease were excluded) The test was positive in eight cases The share of glycemic disturbance was increased thereby from 17 14 % to 40%

## DISCUSSION

No longer than two years after Dupuytren s description Goyrand (quoted by Millesi (6)) drew attention to the family occurrence of D C Millesi (6) in his extensive series found D C simultaneously on the palm and on the sole in 12% and in 2 37 % he found D C together with induratio penis plastica There is no doubt that there is a

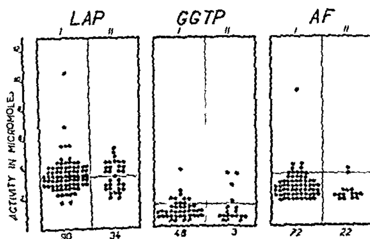



*Fig 1* Highest values of LAP GGTP and AF in patients with D C Values in micromoles. The horizontal line shows the upper limit of normal

Table II *Accompanying diseases in patients with Dupuytren s contracture*

| Disease | Group I | | Group II | | Groups I and II | |
|---|---|---|---|---|---|---|
| | No | Percentage | No | Percentage | No | Percentage |
| Ethylism | 30 | 33 3 | 11 | 31 42 | 41 | 32.8 |
| Ulcus gastroduodeni chron | 17 | 18 8 | 11 | 31 42 | [illegible]8 | 2[illegible] 4 |
| Cholecystitis chron | 9 | 10 0 | 12 | 34 [illegible]8 | 21 | 16 8 |
| Hepatitis epid in anamnesi | 11 | 12.2 | 1 | 2.85 | 12 | 9 6 |
| Diabetes mellitus | 4 | 4 4 | 6 | 17 14 | 10 | 8 0 |
| Cirrhosis hepatis | — | — | 9 | 25 71 | 9 | 7 2 |
| Hepatitis chron | — | — | 3 | 8 50 | 3 | [illegible] 4 |
| Pancreatitis chron | — | — | 1 | 2.80 | 1 | 0 8 |
| Family occurrence of D C | 4 | 26 6 | 2 | 5 71 | 6 | 0 8 |

connection between D C and oth r alterations of the organism not so well known so far especially with metabolic changes namely with carbohydrate symptoms and hepatic lesions There is a very evident coincidence between D C and diabetes mellitus *and between D C and hepatic cirrhosis* especially the alcoholic type Wegmann et al (18) examined patients with D C and found that 48 % of them were alcoholics 7 5 were diabetics and in 5 % diabetes was accompanied by alcoholism Even more conclusive are the observations made by Rhomberg (10) who examined a group of 100 patients and found manifest diabetes in 25 cases and in 27 % the tolbutamide test (TT) pointed to latent diabetes In the next 29 % TT was not normal and in less than one fifth of cases only 19 % TT was normal Together with the disturbance of carbohydrates he found that the average values of free fatty acids and phospholipids were evidently higher in cases of D C than in normal p rsons Nine of his patients suffered from cirrhosis as well The same conclusions concerning carbohydrate changes were made by Siegenthaler (11) who however draws attention to the fact that even in elderly healthy persons there is often abnormal glycemia Frequent hepatic cirrhoses are referred to in the studies of Wegmann and Geiser (16 17) Neuschaefer Rube (7) and others who found D C in 19–66 % of cirrhotic patients Somewhat unusual results were found by Huslarova et al (4) who examining 140 patients with advanced D C who were operated upon did not find a single case of typical hepatic cirrhosis nevertheless changes in liver tests were rather frequent (Prontosil excretory test according to Siede was positive in 28 8 % and there was even an increase of AP in serum in 37 7 %) Bitter (2) on the other hand examining a group of 60 patients with hepatic cirrhosis found almost in 50% (48 3 %) the first stage of D C *even more often than erythema palmare* Pa slack (8) also verified a more frequent occurrence of diabetes in patients with D C and a family accumulation of D C in diabetic families but he nevertheless emphasizes the fact that D C precedes the manifestation of diabetes and that therefore it is not probable that a metabolic disturbance could be the direct cause of D C Rather in his opinion a common genetic factor must be considered responsible for the origin of both diseases Even if D C is not the consequence of a single cause but of a spectrum of causes it seems nevertheless that it is often accompanied by hepatic lesions The frequent coincidence of D C with alcoholism cirrhosis diabetes mellitus and our own observation in our series with chronic ulcers of the stomach and duodenum and of cholecystitis attracted our attention to the extent to which the hepatic lesion was accompani d by enzymatic changes The more so as it is well known that particularly a long lasting ulcer of the stomach or duodenum often causes e g latent hepatopathy which usually is not severe and which even need not be detected by liver biopsy (19) Not having found any outstanding changes in the levels of serum transaminases we followed the activity of excretory enzymes LAP GGPT and AP whose level rises as a result of bile duct obstructions and of cholestasis Statistically highly significant elevation of LAP and GGPT and the

increase of AP even if not statistically significant, assessed in 20 2 % of patients with D C suggest a conspicuous disturbance of enzymes indicating a latent liver injury

## REFERENCES

1 Bessey O A., Lowry O H. & Brock, M J A method for the rapid determination of alkaline phosphatase with five cubic milimeter of serum J biol Chem. 164 321 1946
2 Bitter J Dupuytrenova kontraktura u jaterních cirhos Vnitrni Lek. 12 575 1966
3 Goldbarg J A & Rutenburg, A. M The colorimetric determination of leucine amino peptidase in urine and serum of normal subject and patients with cancer and other diseases. Cancer (Philad) 11 283 1958
4 Huslarová A Sivánova, Z. Peškova, H., Sedlacek J & Škachova, J Die Beziehung der Dupuytrenschen Kontraktur zur Leberzirrhose Gastroenterologia (Basel) 101 32 1964
5 Kulhánek V & Dimov D M A new useful modification for the determination of $\gamma$ glutamyl transpeptidase activity Clin chim. Acta 14 619 1966
6 Millesi H Zur Pathogenese und Therapie der Dupuytrenschen Kontraktur Ergebn. Chir Orthop 47 51 1965
7 Neuschafer Rube R. Dupuytrensche Kontraktur und Leberzirrhose Med Welt. (Stuttg) 9 430 1961
8 Paeslack, V Dupuytrensche Kontraktur und Diabetes mellitus Schweiz. Med Wschr 92 349 1962
9 Reitman, S & Frankel S A colorimetric method for the determination of serum glutamic-oxalacetic and glutamic pyruvic transaminases Amer J clin. Path 28 56 1955
10 Rhomberg, H P Dupuytrensche Kontraktur und innerne Erkrankungen Wien klin. Wschr 79 792, 1967
11 Siegenthaler P Maladie de Dupuytren et pertubation de la glucoregulation. Helv med Acta 31 538 1964
12 *Sigma Technical Bulletin Leucinamino-peptidase* No 250 1963
13 Ševela, M K fotometrickému stanovení serových transaminas. Vnitrní Lék. 4 7_1 1958
14 Ševela, M & Továrek, J Metoda stanovení laktikodehydrogenasy v telních tekutinách Čas. Lek ces. 98 844 1959
15 Vogl A. Zur Atiologie der Dupuytrenschen Kontraktur Med. Klin 6 750 1967
16. Wegmann T & Geiser W Gehauftes Vorkommen der Dupuytrenschen Kontraktur bei chronischem Alkolismus. Schweiz. med Wschr 91 719 1961
17 — Dupuytrensche Kontraktur der Hand als internistisches Problem. Helv med Acta 31 66 1964
18 Wegmann T., Gurtner B & Munz, W Dupuytrensche Kontraktur Diabetes mellitus und chronischer Athylismus. Schweiz. Med Wschr 96 852, 1966
19 Yamagata, T., Wahni, K. Sato S., Ito K., Sanada M., Kumada, H., Matsuda, V., Osato J Yanbe R & Tadaki H. Liver function tests in patients with peptic ulcer Tohoku J exp Med. 90 355 1966.

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# ANKLE JERK ESTIMATION AND THE THYROID FUNCTION IN A HEALTH SURVEY

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*Abstract* The reliability of the ankle jerk test (AJT) as an indicator of thyroid hypofunction has been studied in a health survey A strong correlation was found between the AJT and the results of clinical scoring. The AJT could not be correlated to the serum PBI, cholesterol or the T resin uptake test. As the diagnosis of thyroid hypofunction remains primarily clinical the AJT is suggested as the method of choice for screening purposes as compared to PBI cholesterol and T resin uptake measurements.

Following Chaney s original report in 1924 (4) on the estimation of the Achilles reflex in myxedema several authors have reported on the usefulness of this simple procedure as an aid to evaluate thyroid function (1 5 6 7 8 9 10 11 13 15 16 18) The aim of the present study was to evaluate this method as a screening procedure for hypothyroid states in a health survey

## MATERIAL

From a health survey of randomly selected civil servants in Stockholm, 276 *consecutive subjects were included in* the present study during February 1966 The group consisted of 21? women with a mean age of 41 (range 17–67) years and 64 men with a mean age of 38 (range -4-64) years (Table I)

## METHODS

All subjects were initially examined by one of the authors, and thyroid function was scored on a clinical basis, including the serum cholesterol value and answers to a questionnaire No other thyroid function tests were available to the examiner

The Achilles reflex measurements were performed by a technician using the Lawson kinemometer (Fig. 1) The subjects rested ten minutes prior to the examination The tracings were recorded on a standard electrocardiograph at a paper speed of 0 mm per sec The contraction time of the ankle jerk was measured as the interval between the stimulu and the end of the contraction, i.e the s.d interval (Fig. ) The mean value of two measurements on each side was used as the valu of the ankle jerk test (AJT) Weissbein and Lawson ( 0) give a normal range between 19 and 8 csec. Subjects with values outside this range were further examined with determination of serum protein-bound iodine (PBI) T resin uptake test and re-estimation of the AJT Serum PBI was determined by the alkaline cineration procedure essentially according to Barker et al (3) The T resin uptake test was done by the Trisorb method described by Mitchell et al (1-)

To obtain a control group serum PBI and T resin uptake test were analyzed in 57 consecutive subject at the initial examination

## RESULTS

The results of the initial AJT are given in Fig 3 Acceptable curves for the calculation of values were obtained in 258 cases Of the remaining 18 subjects ten showed absent ankle jerks and in eight subjects technically unacceptable curves were obtained

The mean value for the AJT in the total male group was $24\,6 \pm 0\,3$ (s D 2 0) csec and in the female group $25\,3 \pm 0\,2$ (s D 2 7) csec (Table II) This difference falls short of statistical significance

In order to investigate any difference in the AJT between age and sex groups the males and females were subdivided into two groups the age of 35 being taken as the dividing point (Table II) Females aged 35 years and above were found to have a significantly longer contraction time than the males in the same age group ($p < 0\,001$) and the younger females ($p < 0\,05$) There was no difference between the two male age groups

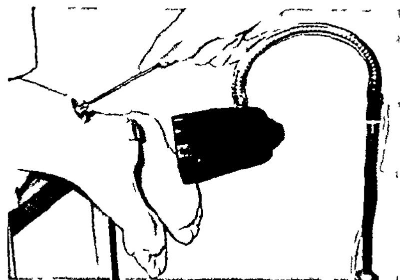


*Fig 1* Sensor head of the kinemometer and magnet attached to the patient's heel

Applying the criteria given by Weissbein and Lawson (20) high values (28 csec and above) were seen in two men and 32 women Of these 34 subjects 25 accepted invitation to re examination within two months from the initial examination The results of the two examinations are given in Table III The initial AJT values and those obtained at re examination differed significantly ($p < 0\,01$) There was a mean decrease of 2 2 csec in the AJT values Out of the 25 subjects with initially lengthened AJT values 15 had fallen within the normal range

Eighteen subjects (one man and 17 women) from the control group were re examined during the same period (Table III) A significant shortening of the contraction time was also seen in this group ($p < 0\,01$) The mean decrease was 1 4 csec

The mean changes in the two groups did not statistically differ at comparison

The initial AJT values could neither be correlated to serum PBI nor to cholesterol levels or resin uptake values

Table I *Age and sex distribution*

| Age (y) | No of men | No of women | Total no of subjects |
|---|---|---|---|
| >55 | 6 | 34 | 40 |
| 46–55 | 16 | 33 | 49 |
| 36–45 | 20 | 37 | 57 |
| 26–35 | 21 | 69 | 90 |
| ≤25 | 1 | 39 | 40 |
| | 64 | 212 | 2 6 |

A normal score was noted in 241 subjects out of whom 18 (7%) had initial AJT values of 28 csec or above Abnormal scores were found in 17 of the 258 subjects A score indicating thyroid hypofunction was present in 15 of these cases, all women out of whom 14 (93%) showed initial AJT values of 28 csec or above A score indicating thyroid hyperfunction was found in two subjects

The mean initial AJT value in the 15 subjects with clinical score indicating thyroid hypofunction was 29 1 ± 2 4 csec (mean ± S D ) The corresponding value in the 241 subjects with normal score was 25 0 ± 2 4 csec This difference was highly significant ($p < 0\,001$)

In the 15 subjects with score indicating thyroid hypofunction the mean PBI (5 4 μg%) was not significantly lower than in 49 subjects from the control group with normal score (5 8 μg %) PBI values 4 5 μg% or lower were noted in four out of the 15 subjects with score indicating thyroid hypofunction and in nine out of 49 subjects with normal score This difference is not significant ($p > 0\,5$)

## DISCUSSION

In a health survey a test for the estimation of thyroid function should be simple accurate and cheap The ankle jerk test satisfies the need for simplicity but its reliability has been questioned (2 14 17)

In this study 14% of subjects with acceptable curves at the initial examination were found to

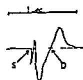



*Fig 2* AJT curve with the S D interval

have values outside the normal range of 19–28 csec as set by Weissbein and Lawson If the upper normal limit is supposed to be the mean + two S D this will be about 28 5 csec for the males and young females in this study Consequently this limit is in agreement with that of Lawson and Weissbein The same limit for the older female group would be around 32 csec In this study it is worth noting that almost all subjects with a clinical score indicating thyroid hypofunction were found in this group

The lowering of the AJT values obtained at re-examination irrespective of whether the initial value was raised or not could be due to seasonal variation It must be pointed out that the first examination was carried out in February whereas the second took place 4 to 7 weeks later Seasonal variations in AJT and serum cholesterol have been described by Thorp (19) according to whom February seems to be a time of relative thyroid hypofunction If it is true that thyroid function varies during the year this has to be taken into consideration when using the kinemometer as a screening aid

The AJT values were strongly correlated to the clinical scores while on the other hand there was a lack of agreement with PBI cholesterol or resin uptake test If it is accepted that thyroid hypofunction is a state that is mainly defined on clinical grounds the results of this study support the use of the AJT in health screenings

The lack of correlation to the other above mentioned laboratory tests can be explained by the fact that neither PBI nor the resin uptake test measures the concentration of free thyroid hormones in the serum Nor will the serum PBI or resin uptake test give any idea of the balance between need and supply of these hormones in the tissues Consequently a peripheral deficit of these hormones could exist with so-called normal PBI values

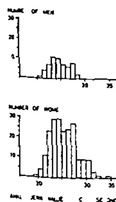


*Fig 3* Distribution of initial AJT values

Table II *Mean initial AJT values (csec) in different age and sex groups*

| Age (y ) | Men | Women |
|---|---|---|
| <35 | 4 8±0 4 | 24 4±0 ? |
| >35 | 4 5±0 4 | 26 3±0 3 |
| <35+ ≥35 | 24 6±0 3 | 25 3±0 2 |

[a] Mean ± S E

Table III *Re examined subjects*

| | Subjects with AJT values ≥ 8 0 csec at the initial examination | Control group |
|---|---|---|
| No of subjects | 5 | 18 |
| Mean age | 45 4±1 0 | 33 4±9 8 |
| No of subjects with | | |
| score 1 (thyroid hypofunction) | 3 | 0 |
| score 2 (suspicion of thyroid hypofunction) | 10 | 0 |
| score 3 (normal thyroid function) | 1 | 18 |
| Initial AJT value csec | 29 6±1 5 | 4 5±1 8 |
| AJT value at re-examination csec | 27 4±2 4 | 23 2±1 8 |
| Decrease of AJT value csec | 2 2±1 8 | 1 4±1 6 |
| PBI μg | 5 3±0 9 | 6 2±1 3 |
| Cholesterol mg | 270±41 | 259±41 |

All values = mean ± S D

## REFERENCES

1 Abraham, A S Atkinson, M & Roscoe B Value of ankle jerk timing in the assessment of thyroid function Brit med J 1 830 1966

2. Andersen, P Achillesreflexens kontraktionsfase som mål for thyreoideafunktionen Nord. Med 79 164 1968
3 Barker S B., Humphrey M J & Soley M H The clinical determination of protein bound iodine J clin. Invest. 30 55 1951
4 Chaney W C. Tendon reflexes in myxedema. J Amer med Ass. 82 2013 1924
5 Ekbom, K Hed R., Herdenstam, C-G P & Nygren A The serum creatine phosphokinas activity and the Achilles reflex in hyperthyroidism and hypothyroidism. Acta med scand 179 433 1966
6 Fogel R. L., Epstein, J A Stopak, J H & Kupperman, H S Achilles tendon reflex test (photomotogram) as a measure of thyroid function. N Y St J Med 62 1159 1962.
7 Gordon, M B The Achilles reflex test (A R.T) in the diagnosis of thyroid dysfunction. Med. Tms (N Y) 90 915 1962.
8 Lambert, E H Underdahl L. O B ckett, S & Mederos, L. O A study of the ankle jerk in myxedema. J clin Endocr 11 1186 1951
9 Lawson, J D The free Achilles reflex in hypothyroidism and hyperthyroidism. New Engl J Med 259 761 1958
10 — The free Achilles reflex as an aid in the diagnosis of thyroid dysfun tion. U.S armed Forces med J 10 16 1959
11 Miles, D W & Surveyor I. Role of the ankle jerk in the diagnosis and management of thyroid disease Brit med J 1 158 1965
12 Mitchell M L Harden A. B & O Rourke M E. The in vitro resin sponge uptake of triiodothyronine I[131] from serum in thyroid disease and in pregnancy J clin. Endocr 20 1474 1960
13 Nordyke R A & Gilbert F I Mass screening for hypothyroidism by the Achilles reflex time Arch environm Hlth 14 8 7 1967
14 Quigley M & Hales, I Achilles tendon reflex in the diagnosis of thyroid disease Med J Aust 2 615 1963
15 Reinfrank R. F., Kaufman, R P Wetstone H J & Glennon J A Observations on the Achilles reflex test. J Amer med Ass. 199 1 1967
16 Ringqvist, I Achillesreflextiden som prov på tyreoidea funktion Paper read at Medicinsk riksstämma, Stockholm 1966
17 Rives, K. L Furth E. D & Becker D V Limitations of the ankle jerk test. Ann. intern Med. 62 1139 1965
18 Sherman, L. Goldberg, M & Larson, F C The Achilles reflex. A diagnostic test of thyroid dysfunction. Lancet 1 243 1963
19 Thorp J M Experimental approach of the problem of disordered lipid metabolism J Atheroscler Res. 3 351 1963
20 Weissbein, A. S & Lawson J D Paradoxical lengthening of muscle contraction time in hypothyroid patients given small doses of l triiodothyronine thyroxine and desiccated thyroid. J clin Endocr 20 479 1960

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# DRUG INDUCED AGRANULOCYTOSIS WITH SPECIAL REFERENCE TO AMINOPHENAZONE

## I Adults

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*Abstract* This is a report of 63 episodes of agranulocytosis in 51 patients during a period of 18 5 years (1950–68) In 54 cases (93%) there was a positive history of drug ingestion, most of those without such history being from the period before 1959 An "idiopathic" case of agranulocytosis is almost entirely a function of inadequate history taking. The frequency of agranulocytosis has greatly increased during the period, the increment being entirely in women. During the years 1950–59 the ratio of men to women was 4 11 in 1960–68 4 44 The mortality has decreased from the 83% of 1950–54 to 13% in 1960–68 being close to zero for patients under 50 years of age and in adequate treatment.

As causative agents, sulphonamides and aminophenazone were the most commonly observed, the estimated incidence for the latter being 1 per 40 000 cures of the drug. A history of the use of chloramphenicol was rather common, but always in combination with other drugs known to cause agranulocytosis. The next most common agents were carbimazole phenylbutazone and thioridazine occasional cases being attributed to carbutamide mephenytoin, thiamazole, phenobarbital barbital amithiozole, thiocarlide metamizol natrium, tetracycline chlorothiazide and chlorpromazine

Following the description by Schultz in 1922 (11) of six cases af agranulocytosis a general interest in this disease was aroused From 1925–34 reports of new cases appeared and it became obvious that the disease had an aetiological relation ship to aminophenazone (Pharmacopoea Nordica called aminopyrine by the United States Pharmacopeia) After 1934 the number of reported cases of agranulocytosis decreased and coincided with the reduced sale of aminophenazone (8) Later however as potent new drugs were introduced more new cases appeared and at present many other drugs are also known to be causative agents of agranulocytosis

In recent years reports of agranulocytosis have been related primarily to new drugs or drugs for which a clear causative relationship has not been previously established The occurrence of agranulocytosis among children and adults under 25 years is very low Wintrobe in 1967 (14) could find only nine substantiated reports of the disorder in children Therefore it might be quite important to determine the current relationship of drugs to agranulocytosis in Finland, where aminophenazone is still sold over the-counter and is used a great deal as an antipyretic especially for children

## MATERIAL

The material was collected from January 1950 through June 1968 from the First and Second Departments of Medicine University of Helsinki and the Department of Medicine University of Oulu and from January 1960 through June 1968 from the Third Department of Medicine University of Helsinki All cases of agranulocytosis during the years 1967 and 1968 were also collected from all hospitals of Oulu and Lappi provinces.

Reported cases fulfilled the following criteria 1) the typical clinical picture of severe illness, sore throat, and fever 2) granulocytes less than 1000/mm³ 3) the bone marrow and peripheral blood showed no evidence of other blood disorders, and erythropoiesis and thrombopoiesis were essentially normal. Cases were excluded in which agents regularly causing marrow depression were utilized, such as X ray or cytostatic drugs.

All drugs used during the three week period prior to the onset of symptoms of agranulocytosis were recorded. The frequency of cases of agranulocytosis was calculated on the basis of the population of the regions examined and also on the sale of aminophenazone

The paediatric series will be reported later

Table II *The fate of patients with agranulocytosis*

| Years | Total no of episodes | No with known etiology | Dead of all cases (No) | Dead of all cases ( ) | Dead of cases with aetiology probably substantiated (No) | Dead of cases with aetiology probably substantiated ( ) |
|---|---|---|---|---|---|---|
| 1950–54 | 6 | 5 | 5 | 83 | 4 | 80 |
| 1955–59 | 9 | 5 | 3 | 33 | 1 | 20 |
| 1960–64 | 24 | 22 | 3 | 13 | 2 | 10 |
| 1965–68 | 24 | 24 | 3 | 13 | 3 | 13 |

Table III *Frequency of association of specific drug (alone and in combination) usage in patients with agranulocytosis*

| Drug | Total no | Only drug used |
|---|---|---|
| *Analgesics* | | |
| *Pyrazolon derivatives* | | |
| Aminophenazone (antipyrine) | 14 | 5 |
| Phenazone (antipyrine) | 5 | |
| Metamizol natrium (dipyrone) | 8 | 2 |
| Phenylbutazone and oxyphenbutazone | 8 | 2 |
| *Others* | | |
| Acetylsalicylic acid and salicylamide | 5 | |
| Codeine | 2 | |
| Phenacetin | 2 | |
| Aethylmorphine | 2 | |
| Paracetamol | 1 | |
| *Chemotherapeutic agents* | | |
| Sulphonamides | 13 | 6 |
| Chloramphenicol | 9 | |
| Tetracyclines | 5 | |
| Streptomycin | 4 | |
| Penicillins | 3 | |
| Erythromycin para aminosalicylic acid iodo-chloroxyquin isoniazide prothionamide each | 1 | |
| Thiosemicarbazides amithiozole thiocarlide each | 1 | |
| *Hypnotics and sedatives* | | |
| Phenobarbital | 5 | |
| Other barbiturates | 6 | |
| Thioridazine | 2 | 1 |
| Chlorproxitene hydroxyzine chlorpromazine pericyazine each | 1 | |
| *Mercaptoimidazole derivatives* | | |
| Carbimazole | 4 | 3 |
| Thiamazole | 1 | 1 |
| *Others* | | |
| Prednisone | 2 | |
| Ethenzamide | 2 | |
| Chlormezanon | 2 | |
| Digoxin | 2 | |
| Carbutamide | 1 | 1 |
| Mephenytoin | 1 | 1 |
| Phenytoin chlorothiazide diphenhydramine caffeine potassium iodide each | 1 | |

the aetiological agent is known has had no effect on prognosis

During the years 1950–59 the mortality was about the same for patients under 60 years of age (5 of 10 or 50 %) than for the older (3 of 5 or 60%) During the years 1960–68 two of the 27 patients (7%) under 60 years of age died while 4 of 21 patients (19 %) over 60 years died Only one patient under 50 years of age died during the 1960–68 period She had received aminophenazone while still in hospital as drug restrictions had not been strict enough

The frequency of association of a specific drug alone or in combination in patients with agranulocytosis is presented in Table III Sulphonamides and aminophenazone are the most common agents observed both alone and in combination Chloramphenicol is the third most common but it has not been used alone in any case and in every case it was administered with another drug also known to cause agranulocytosis As the only drug used carbimazole is the third most frequent The other drugs used alone are phenylbutazone thioridazine carbutamide mephenytoin and thiamazole The drugs stated above can definitely be considered the causative agents of agranulocytosis because they were the only drugs used In addition according to the case histories the following drugs can very probably be considered causative agents amithiozole (case 2) thiocarlide (case 35) metamizol natrium (case 23) tetracycline (case 30) chlorothiazide (case 24) phenobarbital (case 10) barbital (case 10) and chlorpromazine (case 63) Case 10 in which the barbiturates proved to be causative agents deserves a more detailed description

## CASE REPORT

A 45 year-old telephone operator was placed on a combination of phenytoin and phenobarbital in 1954 for

epilepsy In September 1956 she was hospitalized becaus of a gynaecological infection and was treated with peni cillin, streptomycin, phenobarbital atropine and aminophenazone She became more severely ill and had mucosal ulcerations and five days later the white blood cell count was 12 000 with 1 of neutrophils. One week later the neutrophils were absent from the peripheral blood. She was treated with penicillin and prednison (40 mg daily) the remission occurred after two weeks. The patient was subsequently discharged on 150 mg of phenobarbital and 150 mg of caffeine daily to control her epilepsy

In December 1956 the patient developed fever and diarrhoea and was treated with chloramphenicol 1 5 g daily for four days when neutrophils were noted to be absent from the peripheral blood She was treated with penicillin, streptomycin and prednisone (40 mg daily) with a remission beginning after three weeks.

A gallstone was diagnos d by X ray and cholecystec tomy was performed in February 1957 Postoperatively she was given a suppository containing 500 mg of barbital and 50 mg of aethylmorphine on each of four evenings After the third dose she developed a fever and after the fourth dose the absence of neutrophils in the peripheral blood was found On the first two postoperative days the patient rece ved prednisone 30 mg/day this being changed to ACTH 40 units/day on the third day Fol lowing the development of the agranulocytosis the patient was restarted on prednisone 40 mg/day along with anti biotics Remission took place two weeks later

The only drug common to all three episodes of agranulocytosis was a barbiturate (phenobarbital in the first two and barbital in the last) Each episode took place after a large d se of the drug. Another special f ature is the delayed remission (14 to 21 days) after discontinuing of the causative drug and while on predni sone The difference is very striking as compared with other cases treated with corticosteroids most of whom had a remiss on three days after the beginning of treat ment (to be reported in greater detail later)

In the provinces of Oulu and Lappi four cases of agranulocytosis appeared during each of the years 1967 and 1968 As the mean population of 15 years and above in these provinces was 400 000 the total incidence of agranulocytosis was 1 100 000 each year in this population

The sale of tablets containing aminophenazone in Finland was 300 000 packages in 1967 some 12% being sold in the two northern provinces Aminophenazone was the causative agent of agranulocytosis in one case in these provinces in each year which gives an incidence of one case per 40 000 cures" of the drug

## DISCUSSION

We found 63 episodes of agranulocytosis during 18 5 years in the Finnish hospitals studied The incidence during this period appeared to be in creasing but changes at these hospitals such as the area from which they draw patients make a definitive conclusion impossible

The ratio of men to women is 4 11 in 1950–59 which is of the same order as the 1 2 or 3 reported earlier by Plum (8) In contrast during 1960 to 1968 the ratio of men to women was 4 44 or 1 11 The increment of the cases of agranulocytosis has thus been entirely among women

A history of prior ingestion of drugs is positive in 56 cases (89%) Most cases with a negative drug history derive from the first years of the study It is possible that these early findings are due to an inadequate drug history since an association between drugs and agranulocytosis did not occur to most physicians Idiopathic agranulocytosis is thus 11% or less in our series which is greatly different from other observations in which idiopathic agranulocytosis occurred in up to 56 % (3) In our view the incidence of idiopathic agra nulocytosis has disappeared since 1965 as the quest for an accurate drug history has increased

In our material aminophenazone and sulphonamides are the drugs most often associated with agranulocytosis When considered as single drug entities sulphonamides appear to be the most frequent culprit Actually analgesics are most commonly used as combinations and therefore the low incidence of aminophenazone as single entities may give too comforting a view of its guilt in producing agranulocytosis The incidence of agranulocytosis caused by aminophenazone is in our material anyway lower than reported earlier (14) Four other pyrazoline derivatives are also present in our material viz. antipyrine USP (phenazone PhN) dipyrone USP (metamizol na trium PhN) phenylbutazone and oxyphenbutazone

Phenylbutazone and its oxidation product are well known causes of agranulocytosis (14) Dipyrone is present in our material only once but in that case it is probably the cause of the disease The increasing use of dipyrone in the USA has been noted by Huguley (6) where between 1954–64 it has been associated more often with agranu locytosis than aminophenazone Huguley assumes that the physicians in the USA have been unaware of its structural relationship to aminophenazone This is probably true also in Finland esp cially

since the nomenclature in the Pharmacopoea Nordica also fails to suggest this relationship

The appearance of phenazone in our material is fairly frequent To our knowledge this drug has not been previously reported to be considered as the aetiological agent of agranulocytosis

The incidence of sulphonamides as the causative agent in our series is higher than in other published reports Most evidence of the incidence of drugs as the causative agents of agranulocytosis has come from reviews of the literature of isolated cases or small groups of cases This has included numerous authors from different geographical areas Next to the data reported in this paper the best report of cases by one group of investigators from the same area is that published by Larrain and Kahler from Chile in 1964 (7) and in this report there were only three cases out of 56 which appeared to be causally related to the ingestion of sulphonamides In only 22 cases in this report however was the causative agent ever established In the AMA Registry of Blood Dyscrasias up to 1963 sulphonamides were implicated four times less than phenothiazides (6) This registry is a poor source of data to determine frequency since physicians submit reports voluntarily and no effort is made to obtain data on all of agranulocytosis which occur Specific phonamides do not appear as causative agents in r material and to implicate a specific sulphonamide out of a mixture is indeed very difficult

It is of interest that in our series of cases chloramphenicol has been implicated only when given at the same time as other agents known to produce agranulocytosis In the series of Welch et al (13) there was also not one case of agranulocytosis in which the patient received only chloramphenicol Thus it seems to us that chloramphenicol very seldom if ever causes agranulocytosis specifically Rather it appears to be associated with a decrease in several types of formed elements of the blood (13)

The appearance and frequency of mephenytoin and mercaptoimidazole derivatives in our series correspond well to earlier observations However the low incidence of carbutamide and phenothiazide derivatives is surprising Carbutamide is at least in the areas studied the most commonly used oral antidiabetic agent The frequency of phenothiazine derivatives in our list is low although in the AMA Registry of Blood Dyscrasias phenothiazine derivatives especially chlorpromazine are reported as the most common agents causing agranulocytosis Can our results be due to the fact that psychiatric patients using chlorpromazine and other major tranquilizers are treated in psychiatric care units even during complications and do not come to the general hospitals rather than to a true rarity of agranulocytosis in patients ingesting these drugs Two of our three cases are from Oulu from a large psychiatric unit close to the University Medical Center We also cannot state that thioridazine is a more common cause of agranulocytosis than other phenothiazine derivatives

The appearance of thiosemicarbazones in our material is remarkable since their use in Finland is very limited Amithozole as the aetiological agent of agranulocytosis is well known (2) but, as far as we know this is the fist time that thiocarlide has been implicated

In case 10 agranulocytosis developed three times twice in association with the use of phenobarbital and on the last occasion with barbital The possible relationship of barbiturates to agranulocytosis has been speculated upon for many years Watkins in 1933 (12) presented 32 cases of agranulocytosis seen at the Mayo Clinic In 24 cases either aminophenazone or some barbiturate had been taken prior to the onset of the disease. Actually in no case was the use of drugs other than barbiturates satisfactorily established so that in 1934 the Council of Pharmacy and Chemistry declared in their report (9) that no definitive case of granulocytopenia has been reported in which barbiturate alone was responsible Thereafter Hadler (4) reported one case of agranulocytosis repeatedly caused by amobarbital alone To our knowledge our case is the second definitively and repeatedly demonstrated case of barbiturate induced agranulocytosis Since a large dose of drugs was required to cause the agranulocytosis and the remission was delayed in spite of corticosteroid treatment we assume that a direct toxicity was the pathophysiological mechanism in this case rather than an immunological phenomenon

In our series there is one case probably due to chlorothiazide and one to tetracycline Thiazide derivatives are well known as a cause of thrombocytopenia sometimes associated with a slight neutropenia but only a few cases of significant neutropenia have been published (1 10)

The role of tetracycline in agranulocytosis is also unsure but very suspicious (13)

## REFERENCES


1 Chrein M B & Rubin I L. Agranulocytosis secondary to hydrochlorothiazide therapy J Amer med. Ass 181 54 1962

2 Davis, J A Viehman, A. J & Frommeyer W B Acute agranulocytosis during the use of Tibione® Sth. med. J (Bgham, Ala) 45 861 1952

3 Erslev A. J Drug induced bone marrow suppression Penn med. J 66 31 1963

4 Hadler A J Granulocytopenia following barbiturate therapy Report of a case New Engl J Med 222 755 1940

5 Huguley C M Jr Drug induced blood dyscrasia Disease a month. Year Book Medical Publ Chicago 1963

6 Huguley C M Jr Agranulocytosis induced by dipyrone a hazardous antipyretic and analgesic. J Amer med Ass 189 938 1964

7 Larrain C & Kahler F Agranulocytosis Estudio de 56 episodos. Rev med Chile 92 661 1964

8 Plum P Clinical and experimental investigations in agranulocytosis H K Lewis & Co London 1937

9 Report of Council on Pharmacy and Chemistry Relation of amidopyrine and barbituric acid derivatives to granulocytopenia J Amer med Ass 102 183 1934

10 Schotland, M G & Grumbach M M Neutropenia in infant secondary to hydrochlorothiazide therapy with a review of hematologic reactions to thiazide drugs. Pediatrics 31 754 1963

11 Schultz, W Über eigenartige Halserkrankungen. Dtsch med Wschr 48 1495 1922

12 Watkins, C H The possible role of barbiturates and amidopyrine in causation of leukopenic states Proc Mayo Clin. 8 713 1933

13 Welch H Lewis C N & Kerlan, I Blood dyscrasias. A nationwide survey Antibiot. et Chemother (Basel) 4 607 1954

14 Wintrobe M M Clinical hematology 6th ed Lea & Febiger Philadelphia 1967

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# INTENSIVE CARE OF MYOCARDIAL INFARCTION

*A Two-year Experience with 329 Patients*

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*Abstract* An analysis of 3 9 cases with acute myocardial infarction (AMI) treated in an intensive care unit for internal medicine (ICU) during a two-year period is presented The series comprises 44% of all hospitalized AMI cases within this period 191 patients were admitted directly via the emergency department, 138 were transferred from medical wards to the ICU In principle only the most severe cases were admitted to the ICU

The direct and "transfer" groups are compared as to (*a*) the clinical severity of the disease (*b*) delay between onset of symptoms and arrival in hospital, and (*c*) incidence and prognostic effect of shock, congestive heart failure and arrhythmias.

Special attention is directed to cardiac arrest. In the case of primary ventricular fibrillation occurring in the ICU patients the results were very good. In the case of complicating ventricular fibrillation the results were rather good. Half of the 69 patients with ventricular fibrillation left the hospital alive In contrast, the results of resuscitation in asystole were very poor

The results obtained very much support the opinion that the AMI patients need special surveillance and possibilities for qualified inten i e measures during their stay in hospital especially in the early phase of the r disease

In the years 1966–67 a total of 717 cardiological patients were treated in the Intensive Care Unit for internal medicine (ICU) in the Central Hospital of Tampere These cases represented 56 of all patients admitted to the ICU during the period In 329 of these cases acute myocardial infarction (AMI) was diagnosed in 104 coronary heart disease without recent infarction The present p per is a detailed report on the AMI series in question

## MATERIAL AND METHODS

Tab e I gives the distribution of the material w th regard to age and sex. Males were in major ty 3 1 It was possible to t eat in the ICU only the ser ou cases with signs of complications, such as arrhythmias, congesu e heart failure (CHF) and cardiogenic shock. No age restrictions were imposed in admittin patients to the ICU The ICU material, with 3 9 patients, represented only 44 of all AMI cases treated in the hospital during the same period.

Patients with AMI were admitted to the ICU by two different routes, 191 of them were taken dire tly through the emergency department ("direct group) as they showed signs of complications on admission From the general wards 138 patients were transferred to the unit ( transfer group) These were relatively well on admission to the hosp tal but their condition grew worse in the ward or potentially dangerous arrhythmias were observed.

The structure equipment and staff of the ICU are described in another paper (13) The modes of treatment were those generally accepted in the period in question again described in earlier papers (5 13)

## RESULTS

*Severity of disease* The AMI patients treated in the ICU were divided into three groups on the basis of coronary prognostic index (CPI) proposed by Peel et al (12) In the direct" group this was assessed upon arrival in the hospital in the trans fer group on transmission from the ward to the ICU (Table II) Table II shows that the clinical severity of the disease was almost the same in both groups on admission to the ICU In the mild" (CPI 1–8) and "moderately severe" cases (CPI 9–16) mortality was significantly higher among transferred patients than among cases directly admitted In the "most severe" cases (CPI 17–28) no difference in this respect was observed between the two groups

*Delay between the onset of symptoms and admission* Patients of the "direct" group arrived in the

Table I *Sex and age in 329 patients with acute myocardial infarction admitted to the ICU during a two year period*

| Sex and age (y) | No of pats | No of deaths | Mortality ( ) |
|---|---|---|---|
| *Men* | | | |
| <45 | 16 | 1 | 6 |
| 45–54 | 58 | 18 | 31 |
| 55–64 | 89 | 32 | 36 |
| 65 and over | 88 | 35 | 39 |
| Total | 251 | 86 | 34 |
| *Women* | | | |
| <50 | 0 | 0 | 0 |
| 50–64 | 25 | 1 | 4 |
| 65 and over | 53 | 27 | 50 |
| Total | 78 | 28 | 36 |

Table II *Classification of patients according to severity of illness by Peel s criteria*

| | Direct group | | Transfer group | |
|---|---|---|---|---|
| CPI | Distribution ( ) | Mortality ( ) | Distribution ( ) | Mortality ( ) |
| 1–8 | 12 | 5 | 11 | 29 |
| 9–16 | 48 | 14 | 48 | 27 |
| ≥8 | 40 | 61 | 41 | 52 |

hospital considerably earlier than those later transferred to the ICU (Table III) Of the direct cases 71 % arrived in the hospital within 6 hours while only 27 % of the transfer patients gained admission so soon Two thirds of the deaths in the direct group occurred among patients who arrived within 6 hours In the transfer group one third of the deaths occurred among the corresponding cases Fatal late complications were common in the transfer group

*Duration of stay in the unit* and its correlation to mortality can be seen in Table IV In both main groups about half of the survivors were treated for more than five days at the ICU In the direct group 62 % of all deaths occurred within 24 hours of admission Only a small number succumbed after their fifth day in hospital In the transferred patients mortality was also high during the first 24 hours stay at the ICU but deaths calculated from the onset of episode occurred later

Table III *Delay between onset of symptoms and admission to hospital*

| | Direct group | | | Transfer group | | |
|---|---|---|---|---|---|---|
| Delay (h) | No of pats | ( ) | No of deaths | No of pats | ( ) | No of deaths |
| < 1 | 42 | 22 | 18 | 17 | 12 | 6 |
| 1– 6 | 94 | 49 | 23 | 21 | 15 | 12 |
| 7–24 | 29 | 15 | 10 | 16 | 12 | 6 |
| >24 | 21 | 11 | 8 | 80 | 58 | 9 |
| Uncertain | 5 | 3 | 2 | 4 | 3 | 0 |
| Total | 191 | 100 | 61 | 138 | 100 | 53 |

*Prognostic effect of congestive heart failure in AMI* patients is shown in Table V About half of the cases in both main groups showed no clinical signs of CHF One quarter of these patients died however as a result of other fatal complications such as arrhythmias A–V dissociation cardiogenic shock etc Mortality among patients with pulmonary edema was high In transferred patients CHF was associated with death in 52 % In many of these cases CHF developed relatively late

*Cardiogenic shock* with or without congestive failure proved to be as common in the direct as in the transfer group its incidence in both cases being 28 % Thirty (32 % ) of the 93 patients with prolonger cardiogenic shock were discharged alive There was no significant difference in survival rate to be observed between the groups

*Arrhythmias or A–V blocks* were observed in 267 cases an incidence of 81 % (Table VI) The relative prognostic significance of serious dysrhythmias is presented In many cases arrhythmia or block was not the immediate cause of death

Table IV *Duration of stay in the ICU*

| | Direct group | | Transfer group | |
|---|---|---|---|---|
| Duration of stay in the ICU | No of pats | No of deaths | No of pats | No of deaths |
| < 6 h | 22 | 21 | 22 | 22 |
| 6–24 h | 22 | 17 | 16 | 14 |
| 1– 5 d | 72 | 15 | 54 | 10 |
| 6–10 d | 43 | 4 | 30 | 5 |
| 11–15 d | 23 | 1 | 10 | 2 |
| >15 d | 9 | 3 | 6 | 0 |
| Total | 191 | 61 | 138 | 53 |

Table V *Incidence of congestive heart failure and its prognostic effect in 329 patients with acute myocardial infarction*

| | Absence of CHF | CHF | Pulm edema |
|---|---|---|---|
| Direct group | | | |
| Distribution ( ) | 43 | 38 | 19 |
| Mortality ( ) | 27 | 32 | 46 |
| "Transfer" group | | | |
| Distribution ( ) | 49 | 42 | 9 |
| Mortality ( ) | 5 | 52 | 42 |

CHF = congestive heart failure

Table VI *Classification of main arrhythmias in 329 patients with acute myocardial infarction*

| Type of arrhythmia | No of pats. | | Mortality No of deaths | |
|---|---|---|---|---|
| Frequent ventricular ectopic beats | 141 | 43 | 39 | 27 |
| Ventricular tachycardia | 53 | 16 | 25 | 47 |
| 2nd or 3rd degree A–V block | 29 | 9 | 12 | 41 |
| Atrial flutter and or fibrillation | 73 | 22 | ‚8 | 38 |
| Supraventricular tachycardia | 14 | 4 | 3 | 21 |

because other severe complications developed simultaneously In this material the highest mortality rate was associated with ventricular tachycardia Second and third degree A–V block and acute atrial flutter/fibrillation seemed likewise to be bad prognostic signs Supraventricular tachycardia proved a less dangerous arrhythmia

*Cardiac arrest* In patients subjected to resuscitative measures the interval from the onset of symptoms to the first episode of cardiac arrest was calculated (Table VII) In the direct group 59 % of the arrests occurred within 24 hours but as many as 29 % were recorded after the fifth day These latter cases frequently involved multiple complications and the prognosis was poor In the "transfer group only a quarter of the cardiac arrests occurred during the first 24 hours but half of them after the fifth day from the calculated onset of the attack

Cardiac arrests were divided into primary complicating and agonal types according to the criteria prescribed by Lawrie et al (4) An analysis from this point of view is set out in Table VIII A total of 121 patients (37 %) suffered from one or several episodes The location of the patient at the time of a cardiac arrest is shown too

Eighteen of these 20 patients who suffered from primary ventricular fibrillation (VF) inside the hospital survived while all but two of those who developed it outside the hospital succumbed All patients with primary VF in the ICU were discharged alive Fifteen patients out of 34 survived from VF episodes complicated by congestive heart failure and cardiogenic shock One of them lived through 59 separate episodes

The results of resuscitative measures with patients suffering from ventricular standstill were less encouraging Only four out of 49 patients left hospital alive The initial results were not very bad but most cases were lost later In 17 cases asystole was of the agonal type according to retrospective evaluation In these cases there were absolutely no premises for an attempt at resuscitation Intracardiac pacing was attempted on a few occasions but with negative results

In three cases the mechanism of cardiac arrest remained unknown Closed chest cardiac massage alone restored an adequate heart function before any registration of FCG

*Patients with AMI treated only in general wards* were analysed in order to give a picture of the treatment of AMI patients as a whole During a two-year period 1966–67 altogether 740 AMI patients were treated in this hospital 411 patients (56 %) remained in general medical wards throughout their hospitalization 26 % of all AMI patients were admitted directly to the ICU and 18 were

Table VII *Interval between onset of symptoms and first episode of cardiac arrest*

| | 1st–‚4th hour | ‚nd–5th day | 6th–10th day | After 10th day |
|---|---|---|---|---|
| Direct group | | | | |
| No of pats | 38 | 8 | 13 | 6 |
| Distribution ( ) | 59 | 12 | 0 | 9 |
| No of deaths | 20 | 6 | 12 | 3 |
| Transfer group | | | | |
| No of pats | 15 | 13 | 12 | 16 |
| Distribution ( ) | 27 | 23 | 21 | 29 |
| No of deaths | 11 | 10 | 9 | 10 |

Table VIII. *Location of patient at first episode of cardiac arrest Type of arrest and results of resuscitation*

| | ICU | Adjoining, medical ward | Other medical wards | Emergency room | Outside hospital | Total | Discharged alive |
|---|---|---|---|---|---|---|---|
| Ventricular fibrillation | | | | | | | |
| Primary | 5 | 4 | 8 | 3 | 14 | 34 | 20 |
| Complicating | 9 | 8 | 12 | 2 | 3 | 34 | 15 |
| Agonal | 1 | — | — | — | — | 1 | 0 |
| | 15 | 12 | 20 | 5 | 17 | 69 | 35 |
| Ventricular standstill | | | | | | | |
| Primary | 1 | 1 | 2 | 1 | 1 | 6 | 1 |
| Complicating | 14 | 3 | 6 | 2 | 1 | 26 | 3 |
| Agonal | 16 | — | 1 | — | — | 17 | 0 |
| | 31 | 4 | 9 | 3 | 2 | 49 | 4 |
| Cardiac arrest of unknown mechanism | — | — | 3 | — | — | 3 | 1 |
| Total | 46 | 16 | 32 | 8 | 19 | 121 | 40 |

transferred from the general wards The total hospital mortality of infarction patients in 1966–67 was 24%

Among patients who were treated only in general wards the mortality was 8% and was mostly restricted to the highest age groups This patient group was classified according to CPI. 61% of *them were "mild" 36% were "moderately severe"* and only 3% fell in the "most severe" group Congestive heart failure was observed in 13% and cardiogenic shock in 1 5% of these patients

*Deaths in the emergency room* Because of incomplete documentation only patients from 1967 could be analysed. Fourteen patients with AMI died in the emergency room despite resuscitative measures These cases are not included in the material presented above A further 14 dead patients were brought to the emergency room In these 28 cases AMI was verified on autopsy as the reason for the fatal outcome

## DISCUSSION

When comparing the character of the present AMI series with the series of other investigators it must be pointed out that our ICU served only a part (44%) of the AMI patients treated in this hospital in the period 1966–67 A variety of other medical cases had to be treated in addition The admission policy could therefore not be the same as in special coronary care units

*Evaluation of prognosis* Of the numerous methods available for assessing the degree of severity of AMI (e g. 10 12 14 15) the present investigation employed the coronary prognostic index by Peel et al (12)

The examination of mortality figures reveals significant differences between the two groups Of the cases assessed as "mild" and moderately severe" 12% died in the direct" group 27% in the "transfer group It may be concluded that complications were not observed soon enough in the general wards In the absence of adequate monitoring many arrhythmias were unnoted until they led e g. to cardiac arrest Likewise incipient heart failure and cardiogenic shock may well have gone without due attention In the "most severe" AMI cases no difference in mortality rates was to be observed between the two main groups

It seems justified to conclude that the assessment of prognosis on admission or within the first three days is unreliable The CPI used scarcely gives enough weight to arrhythmias

Numerous studies have shown that early *admission* decisively influences the prognosis of the *acute stage as the risk of ventricular fibrillation* is greatest at the onset of an attack (4) The flying squads" in Belfast (8) and the Russian special ambulances in large cities are notable attempts to ensure early admission In our material 22% of the direct group gained admission within one hour and 71% within six hours from the onset of symptoms In the "transfer" group the delay was considerably greater

The reasons for this delay were various the central hospital serves a wide area the possibilities of obtaining speedy medical assistance vary considerably within the area, a number of the patients in question came via smaller local hospitals and finally transport may at times have been unsatisfactory

*Heart failure* In the present ICU series pulmonary edema was observed in 15% CHF in 40% and prolonged cardiogenic shock in 28% of the cases In the series by Killip and Kimball (3) pulmonary edema and CHF were discovered as frequently while Lown et al (6) observed pulmonary edema with equal frequency and CHF more frequently than in our cases Cardiogenic shock was encountered in the present material more frequently than in the two series mentioned above In the assessment of shock strict criteria were followed and the common occurrence of prolonged shock indicates the severity of our cases The fact that one third of the patients with severe cardiogenic shock left the hospital alive shows that this condition is not as hopeless as many authors suggest

*The role of arrhythmias* Prevention, early detection and effective treatment of arrhythmias are some of the most important functions of intensive coronary care Many investigators have pointed out that arrhythmias occur in 70–90% of AMI patients (3 7 9) In the present series the incidence of arrhythmias was 81% Most frequent were ventricular ectopic beats. Ventricular tachycardia has been observed in 15–30 % of AMI patients (3 6 7 9) In this material its incidence was 16% and its prognostic effect was severe In the literature there are divergent opinions as to the significance of ventricular tachycardia (1 6) The incidence of atrial fibrillation or flutter (22 ) was similar (6 7) or greater (9) than described elsewhere Their prognostic significance was not negligible A relatively high mortality rate was also associated with 2nd and 3rd degree A–V block.

*Cardiac arrest and resuscitation* In this study a detailed examination was made of the first episode of cardiac arrest In the "direct group in which the patients arrived quickly at the hospital there were 59 of arrests within 24 hours from the onset of symptoms In the "transfer" group where the admission to hospital was delayed, as many as 50% of the cardiac arrests occurred after the fifth day of the disease

In the present series of 329 AMI cases 69 developed ventricular fibrillation one or several times Thirty five of these patients left hospital alive (51%) Only two of the twenty patients who suffered from primary ventricular fibrillation inside the hospital were lost In both these cases the episode started in the general wards The good results of resuscitative measures may be ascribed to the active participation of our qualified nursing staff

There is reason to mention separately the seventeen cases in which ventricular fibrillation had started outside hospital and resuscitative procedure succeeded in the emergency room Most of these patients also developed other serious complications such as severe brain damage Only two patients recovered In these cases ventricular fibrillation had started in the proximity of the hospital

Our results from the treatment of primary or complicating asystole were not encouraging as only four cases were discharged alive Most authors regard asystole prognostically much more serious than ventricular fibrillation (2 9) Asystole occurs usually in patients with multiple complications and is seldom primary Artificial pacing is advised by several authors (e g 16) in the treatment of a ventricular standstill Our results were poor in the attempted cases

It is *concluded* that the results in the treatment of primary ventricular fibrillation were good if the episodes developed inside the hospital and excellent if they started in the ICU In cases with complicating ventricular fibrillation the results would probably have been better if it had been possible to place all these patients directly in the ICU Asystole occurred in most cases in situations where the patients were critically ill or in agony This may explain the poor results It may be supposed that during this period at least 40 more deaths would have occurred without intensive care facilities Thus without the ICU the total hospital mortality of AMI patients would have risen from the actual 24% to at least 29

The analysis of this material shows that all AMI patients ought to be subjected to continuous surveillance in a coronary care unit or in an intensive care unit in the early phase of their illness

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## REFERENCES

1 A symposium presented by the American College of Cardiology and Presbyterian University of Pennsyl vania Medical Center The current status of intensive coronary care The Charles Press New York 1966
2 Goble A J Sloman G & Robinson J S Brit med J 1 1005 1966
3 Killip T & Kimball J T Amer J Cardiol 20 457 1967
4 Lawrie D M Higgins M R Godman M J Oliver M F Julian D G & Donald K W Lancet 2 523 1968
5 Linko E Koskinen P J Siitonen L & Ruos teenoja R Acta med scand 182 611 1967
6 Lown B Vassaux C Hood W B Fakhro A B Kaplinsky E & Roberge G Amer J Cardiol 20 494 1967
7 MacMillan R L Brown K W G Peckham G B Kahn O Hutchinson D B & Paton M Amer J Cardiol 20 451 1967
8 McNeilly R H & Pemberton J Brit med J 3 139 1968
9 Meltzer L E & Kitchell J R Progr cardiov Dis 9 50 1966
10 Norris R M Brandt P W T Caughey D E Lee A J & Scott P J Lancet 1 274 1968
11 Oliver M F Julian G D & Donald, K W Amer J Cardiol 20 465 1967
12 Peel A A Semple T Wang I Lancaster W M & Dall J L G Brit Heart J 24 745 1962
13 Ruosteenoja R Linko E Koskinen P Hakala T & Kauranen O Nord Med 82 1312 1969
14 Russek H I & Zohman B L Amer J med Sci 225 8 1953
15 Schnur S Circulation 7 855 1953
16 Sutton R Chatterjee K & Leatham A Lancet 2 645 1968

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# RENAL ACIDIFICATION AND HYPERGAMMAGLOBULINAEMIA

## *A Study of Rheumatoid Arthritis*

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*Abstract* Renal acidification has been tested by an ammonium chloride load on hypergammaglobulinaemic and eugammaglobulinaemic patients with rheumatoid arthritis. Both groups had normal urinary acidification The result lends support to the suggestion that hypergammaglobulinaemia per se does not cause impairment of renal acidification.

Recently much interest has been focused on the relationship between renal tubular acidosis (RTA) and hypergammaglobulinaemia RTA has been reported to occur in many conditions with hypergammaglobulinaemia i e Sjogren s syndrome (7 9 12) purpura hyperglobulinaemica (2) idiopathic hypergammaglobulinaemia (7) lupoid hepatitis (8) cryoglobulinaemia (6) and in a familial syndrome with elevated immunoglobulins (13)

It has been stated (12 13) that RTA might be more nearly related to states with high levels of precipitating autoantibodies than to hypergammaglobulinaemia per se

Rheumatoid arthritis (RA) is a disease in which hypergammaglobulinaemia is common while precipitating autoantibodies are infrequent In order to test the possible causal relationship between RTA and hypergammaglobulinaemia we therefore decided to study the urinary acidification of RA patients with and without hypergammaglobulinaemia

## MATERIAL AND METHODS

Twenty four patients with RA and six healthy subjects were studied (Table I)

Fifteen of the RA patients had serum total globulin levels between 3 8 and 5.2 g/100 ml the serum gamma globulin of these patients was 1 89–2 70 g/100 ml. In these patients immunoelectrophoresis revealed an elevation mainly of IgG and/or IgA Analytical ultracentrifugation showed that the 7 0 S fraction was elevated. The rheumatoid factor (Latex test Waaler Rose) was positive in ten of the fifteen patients

Nine RA patients had normal serum globulin levels. The immune globulins and the ultracentrifugal fractions were essentially normal in this group The rheumatoid factor was positive in six of the nine patients.

Precipitins to DNA were absent in all the patients.

Only subjects with a normal glomerular filtration rate (endogeneous creatinine clearance >70 ml/min) were selected. All patients had a urinary osmolarity exceeding 600 mOsm/l after overnight fluid deprivation Serum sodium and potassium were normal in all subjects.

*Investigation of renal acidification*

The renal excretion of acid was studied essentially using the short test of urinary acidification described by Wrong and Davies (14) All drugs were interrupted 48 hours prior to the test. Patients treated with corticosteroids or diuretics were excluded. All subjects were kept on an ordinary hospital diet

The voided urine of each subject was collected at two-hour intervals from 7 a.m. to 7 p m Immediately after voiding the urine specimens were put in sealed tubes and frozen to −20 C At 9 a.m. the subjects were given ammonium chloride 0 1 g per kg of body weight as ordinary non-enteric coated tablets Urinary pH was measured using a model 27 Radiometer pH meter Urinary titratable acidity was measured by titrating the urine samples to pH 7 4 with N 0 sodium hydroxide Urinary ammonium was measured by the method of Chaney and Marbach (1)

## RESULTS

The results of the acidification tests are shown in Table II and Fig 1

In all subjects including controls and patients with RA the urinary pH decreased to at least 5 20 This is in agreement with the results obtained in normal subjects by Wrong and Davies (14) and other authors (2 7)

Table I *Clinical and laboratory features*

| Pat no | Diagnosis | Age (y) | Sex | GFR (ml/min) | Serum globulin (g/100 ml) Total | Gamma | RA factor Latex test | Waaler-Rose | Immuno globulins A | G | M | Ultracentrifugal fractions (g/100 ml) 4 5 S | 7 0 S | 18 0 S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Rheumatoid | 63 | ♂ | 82 | 4 9 | 2 42 | + | 64 | ↑ | ↑ | N | 5 9 | 2 3 | 0 2 |
| 2 | arthritis | 50 | ♀ | 104 | 3 9 | 1 89 | − | 32 | ↑ | ↑ | N | 5 0 | 2 0 | 0 3 |
| 3 | with | 58 | ♀ | 134 | 4 4 | 2 28 | + | 128 | N | ↑ | N | 5 5 | 2 0 | 0 3 |
| 4 | hyper | 44 | ♀ | 136 | 4 3 | 2 45 | − | 0 | ↑ | N | N | 5 7 | 3 2 | 0 3 |
| 5 | gamma | 44 | ♂ | 83 | 4 1 | 2 07 | + | 1000 | ↑ | ↑ | N | 5 2 | 1 9 | 0 2 |
| 6 | globulinaemia | 44 | ♀ | 131 | 3 9 | 1 99 | + | 128 | N | ↑ | N | 5 3 | 2 5 | 0 4 |
| 7 | | 22 | ♀ | 105 | 5 2 | 2 70 | − | 0 | ↑ | ↑ | ↑ | 5 0 | 2 3 | 0 2 |
| 8 | | 53 | ♀ | 99 | 4 9 | 2 23 | − | 0 | ↑ | ↑ | N | 5 5 | 2 8 | 0 4 |
| 9 | | 28 | ♀ | 120 | 4 8 | 2 29 | + | 250 | ↑ | N | N | 5 2 | 2 5 | 0 3 |
| 10 | | 45 | ♀ | 115 | 4 1 | 2 29 | + | 1600 | N | N | N | 4 7 | 2 2 | 0 3 |
| 11 | | 24 | ♀ | 79 | 3 8 | 1 91 | − | 0 | N | ↑ | N | 5 3 | 1 6 | 0 3 |
| 12 | | 25 | ♀ | 108 | 4 1 | 2 02 | + | 7000 | ↑ | ↑ | N | 5 7 | 1 9 | 0 3 |
| 13 | | 54 | ♀ | 84 | 4 1 | 2 67 | + | 250 | ↑ | ↑ | N | 5 2 | 2 0 | 0 2 |
| 14 | | 52 | ♀ | 94 | 4 6 | 2 41 | + | 0 | N | ↑ | N | 5 2 | 2 1 | 0 2 |
| 15 | | 54 | ♀ | 113 | 4 6 | 2 17 | − | 0 | ↑ | ↑ | N | 4 8 | 2 2 | 0 3 |
| 16 | Rheumatoid | 32 | ♀ | 103 | 2 9 | 1 28 | − | 16 | N | N | N | 5 8 | 1 3 | 0 3 |
| 17 | arthritis | 45 | ♀ | 113 | 3 5 | 1 33 | + | 250 | N | ↑ | ↑ | 5 1 | 1 2 | 0 4 |
| 18 | with | 40 | ♀ | 82 | 3 0 | 1 06 | − | 0 | N | N | N | 6 4 | 0 9 | 0 3 |
| 19 | eugamma | 35 | ♀ | 74 | 3 3 | 1 27 | − | 0 | N | N | N | 5 6 | 1 0 | 0 3 |
| 20 | globulinaemia | 49 | ♀ | 119 | 3 8 | 1 25 | + | 64 | ↑ | N | N | 5 7 | 1 2 | 0 2 |
| 21 | | 19 | ♀ | 77 | 3 3 | 1 51 | + | 500 | N | ↑ | N | 4 9 | 1 3 | 0 3 |
| 22 | | 50 | ♂ | 122 | 2 9 | 1 07 | + | 1000 | N | N | N | 5 2 | 1 0 | 0 2 |
| 23 | | 35 | ♀ | 92 | 2 8 | 1 02 | − | 0 | N | N | N | 5 3 | 1 3 | 0 3 |
| 24 | | 44 | ♂ | 106 | 3 1 | 1 06 | + | 1000 | N | N | N | 6 0 | 1 0 | 0 3 |
| 25 | Controls | 47 | ♂ | 72 | 2 4 | 0 69 | | | | | | | | |
| 26 | | 30 | ♀ | 173 | 2 5 | 1 04 | | | | | | | | |
| 27 | | 62 | ♀ | 146 | 2 4 | 0 63 | | | | | | | | |
| 28 | | 54 | ♀ | 120 | 2 4 | 0 93 | | | | | | | | |
| 29 | | 48 | ♀ | 131 | 2 8 | 1 02 | | | | | | | | |
| 30 | | 54 | ♀ | 136 | 2 6 | 0 85 | | | | | | | | |

The excretion of titratable acidity increased to at least 3 79 mEq/120 min (31 5 $\mu$Eq/min) in the control group to at least 3 78 mEq/120 min (31 3 $\mu$Eq/min) in the eugammaglobulinaemic RA patients and to 2 82 mEq/120 min (23 5 $\mu$Eq/min) in the hypergammaglobulinaemic RA patients These values are within the normal values of Wrong and Davies (14) The mean of the highest rate of titratable acidity excreted per two hours was 4 97 5 08 and 4 15 in the controls and RA patients respectively The difference of the means is not statistically significant

The excretion rate of ammonium was at least 44 mg/120 min (20 3 $\mu$Eq/min) in the controls 51 mg/120 min (23 5 $\mu$Eq/min) in the eugamma globulinaemic RA patients and 45 mg/120 min (20 8 $\mu$Eq/min) in the hypergammaglobulinaemic RA patients The respective means of the highest ammonium excretion rate per two hours were 76 3 82 1 and 71 6 mg The difference of the means is not statistically significant The ammonium excreted was much lower in this series than has been reported before (2 7 14) This is probably because the method of Chaney and Marbech (1) is more specific than the previously used microdiffusion method of Conway (4) The immediate refrigeration that we used also prevents the release of ammonium from urinary urea and amino acids

There was no correlation between the excretion of titratable acidity or ammonium on the one hand and the glomerular filtration rate (GFR) or levels or types of hypergammaglobulinaemia on the other

## DISCUSSION

The frequency of renal tubular acidosis in hypergammaglobulinaemic conditions has been shown to be surprisingly high Morris and Fudenberg (7) studied 22 patients with hypergammaglobulinaemia

Table II *Results of urinary acidification test*

| Pat. no | Diagnosis | Urinary acidification: Min mal pH | Maximal titratable acid (mEq/2 h) | Maximal $NH_4^+$ (mEq/2 h) | Mean ± S D: Minimal pH | Maximal titratable acid | Maximal $NH^+$ |
|---|---|---|---|---|---|---|---|
| 1 | Rheumatoid | 4 85 | 3 97 | 45 | | | |
| 2 | arthritis | 4 85 | 4 78 | 75 | | | |
| 3 | with | 4 95 | 2 99 | 67 | | | |
| 4 | hyper | 4 65 | 5 14 | 73 | | | |
| 5 | gamma | 4 70 | 6 53 | 60 | | | |
| 6 | globulinaemia | 5 00 | 2 82 | 69 | | | |
| 7 | | 4 70 | 2 87 | 50 | | | |
| 8 | | 4 85 | 3 64 | 47 | | | |
| 9 | | 4 95 | 3 69 | 103 | | | |
| 10 | | 4 50 | 4 95 | 100 | | | |
| 11 | | 4 90 | 3 07 | 85 | | | |
| 12 | | 4 85 | 4 52 | 70 | | | |
| 13 | | 4 85 | 4 54 | 75 | | | |
| 14 | | 4 65 | 5 00 | 91 | | | |
| 15 | | 4 70 | 3 68 | 65 | 4 78±0 15 | 4 15±1 01 | 71 6±5 4 |
| 16 | Rheumatoid | 4 65 | 3 78 | 64 | | | |
| 17 | arthritis | 4 80 | 4 67 | 64 | | | |
| 18 | with | 4 60 | 5 05 | 93 | | | |
| 19 | eugamma | 4 95 | 6 25 | 103 | | | |
| 0 | globulinaemia | 4 85 | 4 85 | 104 | | | |
| 21 | | 5 00 | 4 50 | 86 | | | |
| 22 | | 4 80 | 5 67 | 56 | | | |
| 23 | | 4 65 | 4 18 | 51 | | | |
| 24 | | 4 95 | 6 85 | 118 | 4 80±0 38 | 5 08±0 93 | 82 1±2 7 |
| 25 | Controls | 4 90 | 6 46 | 108 | | | |
| 6 | | 4 50 | 5 86 | 106 | | | |
| 27 | | 4 85 | 4 14 | 70 | | | |
| 28 | | 4 85 | 4 42 | 44 | | | |
| 29 | | 4 80 | 5 13 | 81 | | | |
| 30 | | 5 0 | 3 79 | 48 | 4 85±0 24 | 4 97±0 95 | 76 3±24 7 |

of various aetiology Twelve of their patients had impaired urinary acidification In eight of them definite renal disease was indicated by low glomerular filtration rates and/or proteinuria although no azotaemia was present These authors consider that a causal relationship exists between hypergammaglobulinaemia and RTA, although no correlation between an increased specific globulin component and impairment of renal excretion of acid was observed They discuss the possibility of an underlying vascular alteration which in view of the diseases of many of their patients seems very likely

Six out of twelve patients with Sjogren s syndrome studied by Talal et al (12) had signs of RTA Five of these six patients had subnormal glomerular filtration rates and/or histopathological changes as other signs of renal disease Five of the patients with RTA had purpura a probable sign of a vascular alteration The same authors found significantly more precipitating antibodies in the sera of patients with the sicca syndrome and renal disease including RTA than in those without renal affection

Shearn and Tu (9) studied a patient with Sjogren s syndrome and RTA The patient had an adult Fanconi syndrome The glomerular filtration rate was low and renal biopsy revealed mild glomerular changes and lymphocytic infiltration of the stroma The lesion probably had an immunopathological basis

Wilson et al (13) described three patients with RTA having immunoglobulin abnormalities which also occurred in their relatives One of them had the sicca syndrome the second had rather severe renal insufficiency while the third had a normal renal biopsy finding The aetiology of the RTA could not be defined although it was considered

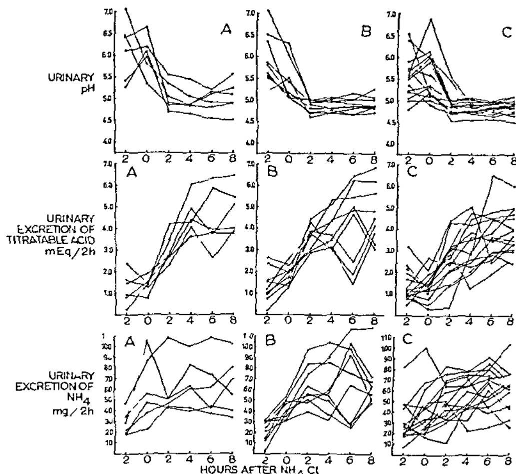



*Fig 1* Urinary excretion of acid after administration of ammonium chloride 0 1 g/kg body weight *A* control subjects *B* rheumatoid arthritis eugammaglobulinaemia *C* rheumatoid arthritis hypergammaglobulinaemia

to be acquired and causally related to the elevated immunoglobulins The probability of autoimmune disease was discussed

Cohen and Way (2) report two patients with purpura hyperglobulinaemica and RTA Renal biopsy performed in one of the patients was normal The same authors add a case of hyper gammaglobulinaemia and RTA not studied by themselves The patient had evidence of renal damage the lymphocytic infiltration in the kidney possibly being a sign of some immunological lesion

One case of hypergammaglobulinaemia and RTA was included in the series of Read et al (8) dealing with so-called lupoid hepatitis The pa tient had additional symptoms lupus erythema tosus of the skin proteinuria and a positive LE factor

Several reports deal with the occurrence of RTA as part of the Fanconi syndrome in mye lomatosis (3 5 10 11) It has been proposed that in these cases the paraprotein is filtered through the glomeruli and on subsequently being reab sorbed in the proximal tubular cells it causes tubular damage (3)

In several of the instances of hypergamma globulinaemia and RTA hitherto discussed there has been evidence of generalized impairment of renal function In some cases this might have resulted from the underlying disease cases of sarcoidosis tuberculosis and coccidioidomycosis treated with amphotericin belong to this group In the case of Sjogren s syndrome hypergamma globulinaemic purpura lupoid hepatitis and fa milial hypergammaglobulinaemia the presence of multiple precipitating antibodies strongly suggest an immunological mechanism In myelomatosis some other kind of tubular damage caused by the paraprotein is probably responsible No relation ship between RTA and the degree or type of

hypergammaglobulinaemia has been established It thus seems impossible to relate the hypergammaglobulinaemia per se to the occurrence of RTA

The present investigation was performed on patients with rheumatoid arthritis in whom renal function was normal and no other systemic manifestations were present In the selection of the patients care was taken to avoid interfering drugs or electrolyte disturbances It was shown that under these circumstances fifteen patients with hypergammaglobulinaemia responded normally to the acid load of ammonium chloride

The result of this study lends indirect support to the suggestion that high serum gammaglobulin levels per se are not related to impairment of renal acidification

## REFERENCES


1 Chaney A L & Marbach E P Modified reagents for determination of urea and ammonia. Clin Chem. 8 130 1962.

2. Cohen, A & Way B J The association of renal tubular acidosis with hypergammaglobulinaemic purpura. Aust. Ann. Med. 11 189 1962.

3 Constanza D & Smoller M Multiple myeloma with the Fanconi syndrome Study of a case with electron microscopy of the kidney Amer J Med 34 125 1963

4 Conway E J Microdiffusion anlysis and volumetric error 3rd ed C Lockwood London 1950

5 Engle R L Jr & Wallis L A Multiple myeloma and the adult Fanconi syndrome I Report of a case with crystal like deposits of the kidney Amer J Med 22 5 1957

6 Lospalluto J Dorward, B Miller W & Ziff M Cryoglobulinemia based on interaction between a gamma macroglobulin and 7 S gamma globulin. Amer J Med 32 142 1962

7 Morris R C Jr & Fudenberg H H Impaired renal acidification in patients with hypergammaglobulinemia. Medicine (Baltimore) 46 57 1967

8 Read A E Sherlock, S & Harrison C V Active juvenile cirrhosis considered as part of a systemic disease and the effect of corticosteroid therapy Gut 4 378 1963

9 Shearn, M A. & Tu, W H Nephrogenic diabetes insipidus and other defects of renal tubular function in Sjogren s syndrome Amer J Med 39 312, 1965

10 Short, I. A. & Smith J P Myelomatosis associated with glycosuria and aminoaciduria Scot. med J 4 89 1959

11 Sirota M A & Hamerman D Renal function studies in an adult with the Fanconi syndrome Amer J Med 16 138 1954

12. Talal N Zisman E. & Schur P H Renal tubular acidosis glomerulonephritis and immunologic factors in Sjogren s syndrome Arthr and Rheum 11 774 1968

13 Wilson I D Williams R C & Tobian L. Renal tubular acidosis Three cases with immunologic abnormalities in the patients and their kindreds Amer J Med 43 356 1967

14 Wrong O & Davies H E. F The excretion of acid in renal disease Quart J Med 28 259 1959

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# EFFECT OF VITAMIN $B_6$ UPON GASTROPRIVAL CENTRAL NERVOUS SYSTEM DEGENERATIONS III[1]

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*Abstract* Vitamin $B_6$ administered parenterally has a curative effect upon the chronic CNS degenerations in pig caused by total resection of the gastric fundus This effect is striking and demonstrable clinically as well as morphologically

Of the members of the vitamin B complex studied so far only vitamin $B_6$ has affected chronic CNS degenerations In totally gastrectomized puppies (8) and pig (9) permanent parenteral administration of this vitamin has entirely prevented the post operative appearance of the degenerations

In the present study an investigation was made as to whether this vitamin also had a curative effect upon full blown long standing degenera tions in this case caused by fundal resection

Since the fundus is the sole aetiological factor in the degenerations (10) its removal is equivalent in this respect to total gastrectomy

## EXPERIMENTAL ANIMAL

Pig, Danish land race (no 165) ♂ aged six weeks when received and operated upon three weeks later then weigh ing 20 kg, and 76 cm in length Elective resection of the fundus was performed in the usual way with end to-end anastomosing of the cardia and pylorus which were left The experimental conditions, including the diet and the terminal decreasing food intake were as described previ ously Faeces normal formed. The pig was killed by exsanguination under anaesthesia thirteen months later

### *Clinical changes*

*First observation period* A postoperative spontaneous course of the disease through the first nine months Gradually the pig developed the usual experimental gastrogenic endogenous chronic symptom complex (Petri) characterized, inter alia by greater stunting of growth emaciation skin and hair changes, anaemia as well as several clinical CNS changes (7) At last death appeared to be imminent. The weight was 50 kg and length 100 cm.

*Second observation period* During the next four months the pig was treated with vitamin $B_6$. The vitamin used was pyridoxine hydrochloride (Benadon® Roche) dosage 50 mg parenterally every 3 days throughout the period.

Already within the first month after the institution of the treatment the pig showed steady improvement of the CNS changes The spasticity pareses and ataxia were rapidly decreasing, apathy and torpidity reduced, vision improved and hypersensibility subsiding. Its posture and gait were much better its back became less bent, and there was only slight slipping of the hind legs. In be haviour the pig was more normal and calmer During the next three months there was further clinical improve ment of the CNS symptoms. At the conclusion of the treatment period the pig was moving about in a fairly natural and uninhibited manner However the normal condition had not been completely restored when the pig had to be killed for lack of space

The other clinical components of the gastrogenic symp tom complex, however remained completely unaffected by the vitamin therapy This also applies to the post operative disappearance of antipernicious principle in the liver (6) Terminally the body weight was only 35 kg and the length 105 cm.

### *Post mortem changes*

1 The external appearance as well as the gross and microscopic findings in the organs corresponded to the previous, constant findings in totally gastrectomized or totally fundus-resected pigs.

The description of the microscopic findings in the central nervous system refers here only to the motor anterior horn cells of the cervical cord as a paradigm. Preparation fixation in formalin, paraffin sections, staining by the method of Einarsson

The nerve cells were fairly uniform in size as a rule medium-sized regularly shaped, well-defined fully granu lated

[1] Read, in an abbreviated form, by Svend Petri to the Annual Meeting of the Scandinavian Neuro-pathological Association in January 1969 Lund, Sweden.



*Figs. 1 and 2* The most severe clinical CNS changes in the fundus-resected pigs at the end of the primary nine-month observation period, without treatment.

The nucleus was of medium size central, rounded, with distinct contours and nucleolus, but partially with increased chromatin content. A smaller proportion of the nerve cells were degenerated to an extent corresponding to the mildest degrees of Nissl's primary irritation. Nissl's severe ganglion cell disease was entirely absent as far as the large, pale, ill-defined, almost agranular and anuclear cells were concerned. Dark, shrunken, "sclerotic" varieties were exceptional findings. Some of the nerve cells could be designated as normal.

The appearance of the nerve cells in the experimental pig was compared with that found in four of our previously fundus-resected, but untreated pigs. The length of observation period and site of tissue were identical.

All nerve cells in these pigs were degenerated at the site of Nissl's primary irritation and Nissl's severe ganglion cell disease. This severe and irregularly mixed picture represented the usual characteristic finding after fundal resection (as well as after total gastrectomy).

It is thus apparent that a striking difference has been found in the appearance of the nerve cells between the treated pig and untreated fundus-resected pigs.

The treatment with vitamin B seems to have entailed (a) an arrest of the progression of the postoperative de towards the severest degrees, preventing terminal necrobiosis, (b) general regression of the degenerations in the remaining cells towards the mildest degrees, and (c) partial restitution to the normal state.

## RECAPITULATION AND COMMENT

From our previous experiment (9) and the present experiment on pigs it is *apparent that the gastrogenic degenerations of the CNS respond electively* to vitamin $B_6$. Partly the *onset* of the degenerations is *completely* prevented and partly the full-blown, chronic degenerations respond to the treatment. The degenerations must be characterized as experimental endogenous, achlorhydric avitaminosis $B_6$.

Between the pigs of the two experiments *there* are differences in respect to age at the institution of vitamin therapy and size of dosage.

In the preventive experiment the pig was less than *two months of age*, far from being full-grown, while in the present curative experiment it was eleven months of age, adult, but *greatly* stunted in growth.

The parenteral dose of pyridoxine hydrochloride was in the previous experiment 7 mg once weekly, later 10 mg twice weekly, and in the present experiment—arbitrarily selected—50 mg every three days. This gives a daily dose per kg body weight—varying according to the postoperative changes in growth—of 0 023–0 065 and 0 29–0 41 mg.

The fact that the CNS was not fully restored in the present experiment must be *due to the rather* short treatment period, an insufficient dose or a not fully reversible postoperative cellular damage.



Fig. 3 Very striking clinical improvement of the CNS changes in the fundus-resected pig at the end of the secondary four-month observation period, with parenteral permanent vitamin $B_6$ therapy.

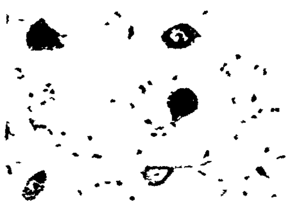


*Fig 4* The nerve cells (in the anterior horn of the cervical cord as paradigm) at the end of the treatment period. The progression of the degenerations has stopped, and at the same time there is a general regression towards the mildest degrees or normal appearances.



*Fig 5* The nerve cells (same site as Fig 4) in previously fundus-resected, untreated pig followed for long periods. All cells severely or very severely degenerated.

As experiments of this nature have not been performed previously there is no basis for direct comparison

However certain statements may serve for an indirect assessment The daily requirement of a normal pig for vitamin $B_6$ in the feed is 0 05–0 1 mg/kg (1 4 5) and in the standard diet during vitamin experiments 0 2 mg/kg (12)

Full blown clinical changes of the nervous system in pigs whose diet is lacking in vitamin $B_6$, viz exogenous avitaminosis have been treated orally by pyridoxine 0 01–0 2 mg/kg daily exceptionally preceded by one i v injection of 10 or 110 mg or else i m injection of 0 02 mg/kg These therapeutic experiments are considered inadequate too few and as a rule too brief and highly varying in effect (2 3 11 12 13)

In one case an effect upon the nerve cells in a spinal ganglion is said to have been observed In return this was the sole result of very prolonged oral treatment (3 $^3/_4$ months) of the avitaminosis by pyrodoxine in a daily dose of up to 0 5 mg/kg (2)

Considering the difference between oral and parenteral administration of the vitamin and the duration of the treatment the effective doses used by us must be said to be moderate or rather large

Our experiments concerning the causation of the CNS degenerations have so far demonstrated two aetiological factors viz the area of the fundal mucosa and vitamin B

That the gastric surgery could have altered the absorption of the vitamin $B_6$ content of the food in the intestinal canal does not at present seem to be the only likely pathogenetic explanation

## ACKNOWLEDGEMENT

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## REFERENCES


1 Cunha T J Swine feeding and nutrition Inter science Publ New York 1957
2 Follis, R. H & Wintrobe M M J exp Med 81 539 1945
3 Hughes, E. H & Squibb R. L. J Animal Sci 1 320 1942.
4 Moustgaard, J Møller P & Thorbek, G Under søgelser over B vitaminernes betydning for svin. V 258 beretning fra forsøgslaboratoriet, Statens Husdyrbrugsudvalg. August Bangs Forlag Copenhagen 1952.
5 Møller P F Studier over vitamin B gruppen Dansk Videnskabs Forlag, Copenhagen 1953
6 Petri S Acta med scand. 185 339 1969
7 Petri, S Nørgaard, F Trautner K & Kiær W Acta med scand 114 184 1943
8 — Acta med scand. 117 90 1944
9 Petri S Nørgaard, F & Kiær W Acta med scand 119 356 1944
10 Petri S & Petri, C Acta med scand 185 453 1969
11 Swank R. L. & Adams R D J Neuropath exp Neurol 7 274 1948
12 Wintrobe M M Follis, R H Jr Alcayaga, R. Paulson M & Humphreys, S Bull Johns Hopk Hosp 72 1 1943
13 Wintrobe M M Miller H M Follis R H Jr Stein H J Mushatt C & Humphreys S J Nutr 24 345 1942

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# RHEUMATOID ARTHRITIS TERMINATING IN PLASMOCYTOMA

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*Abstract* Five patients with rheumatoid arthritis and myeloma are described A typical case of rheumatoid arthritis and benign gammopathy is also reported In all of the patients with myeloma the joint disease was considered to be primary rheumatoid arthritis The cases are discussed against the background of other plasma cell dyscrasias in rheumatics The present cases seem to argue in favour of the view that during the course of chronic rheumatoid arthritis with protracted irritation of the immune system plasma cell diseases are likely to develop During the progression of multiple myeloma the patients joint symptoms abated and the titre of rheumatoid factor in the serum dropped In some of the cases cytostatic treatment of the multiple myeloma may have been of significance for the remission of the joint symptoms

Multiple myeloma is associated with symptoms from the locomotor system The bone changes in myeloma result in pain in the back and often in the shoulder joints as well Myelomatosis may moreover produce high values for uric acid in the blood and symptoms of gout (8) If many joints are involved the clinical picture of multiple myeloma may be confusingly like that of rheumatoid arthritis Patients are sometimes treated for the latter disease for years before myeloma is diagnosed The group of diseases today called rheumatoid arthritis will probably in the future be divided on aetiological grounds into many distinct groups Hyperplasia of the lymphoid system is one of the characteristics of rheumatoid arthritis and plasmocytosis is frequently seen in this disease Can multiple myeloma present as rheumatoid arthritis or do the plasma cells turn malignant during the course of rheumatoid arthritis?

Our clinical observations seem to argue in favour of the view that multiple myeloma may develop in an organism affected by rheumatoid arthritis

## MATERIAL

In 1967 and 1968 three patients with progressive rheumatoid arthritis and multiple myeloma as a severe concomitant disease were treated at the Fourth Department of Medicine On scrutiny of the records of the Rheumatism Foundation Hospital Heinola from 19 8–1968 two similar cases were found These five cases and a case of rheumatoid arthritis showing marked plasma cell proliferation and a monoclonal protein peak but no definite signs of myeloma are described below

## CASE REPORTS

### *Case 1*

A farmer born in 1899 At the end of the 1950 s joint symptoms developed symmetrically in the wrists and knee joints A definite diagnosis of rheumatoid arthritis was made in 1960 and the patient was given ambulatory treatment consisting of salicylates steroids gold and phenylbutazone

At the end of 1964 pain in the muscles of the upper arms developed and the general condition deteriorated At the beginning of 1965 the ESR was elevated ad 147 mm hypochromic anaemia was observed Hb was 8 8 g/100 ml and the leukocyte count was 5400 Serological tests for rheumatoid arthritis were markedly positive Waaler Rose being 700 and latex fixation + + At this stage there were however no noteworthy subjective joint symptoms The patient had proteinuria ad 2 g l but the renal function was normal serum creatinine was 0 9 mg 100 ml The total serum proteins were 7 7 g 100 ml and electrophoresis revealed monoclonal M components between the beta and gamma fields, constituting 30 of the total proteins (Fig 1) Immunoelectrophoresis showed a marked increase of IgA and ultracentrifugation gave a sedimentation constant of 7 S for the M component fraction Bence Jones protein was demonstrated in the urine and bone marrow aspirate contained about 50 plasma cells a considerable proportion of which were pathological X ra s revealed no skeletal signs of myelom but destruction typical of rheumatoid arthritis was observed in the wrists knee joints and basal toe joints

Melfalan therapy was started but had to be discontinued after three months owing to haematological complica

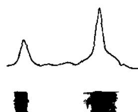


*Fig 1* Electrophoretic pattern of serum proteins in case 1

tions in the form of leukopenia thrombocytopenia and accentuated anaemia The treatment was continued with glucocorticoids

While the plasma cell dyscrasia progressed the joint symptoms improved clinically and subjectively and serological remission was noted the Waaler Rose titre was 450 in Aug 1965 120 in Jan 1966 60 in Jan 1967 and 0 in March 1967 From the beginning of 1967 the patient showed steadily progressing proteinuria and anaemia which could only be controlled by repeated transfusions of blood In Nov 1967 radiolucent foci typical of myeloma were observed in the skull In spite of losses in the urine of 40 g/l the serum protein value rose to 11 7 g/100 ml with about 50 paraproteins The renal function deteriorated and the patient expired in March 1968

*Autopsy findings*

Macroscopically many vertebrae showed myelomatous destruction and active bone marrow was seen in the diaphyses of the long bones Moderate splenomegaly (380 g) was present but no definite lymphoid hyperplasia

Microscopic investigation revealed marked predominance of myeloma cells in the bone marrow Moreover these cells had infiltrated the parenchymatous organs the liver and spleen in particular Renal specimens exhibited changes typical of myelomatous kidney and inflammatory changes mainly resembling those of chronic pyelonephritis No signs of amyloidosis were found

Synovial specimens from the knee joints showed proliferation and chronic inflammatory changes consistent with rheumatoid arthritis No amyloid deposits were observed

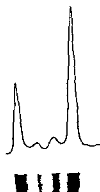


*Fig 2* Electrophoretic pattern of serum proteins in case 2

*Comment*

It may be considered established that the patient suffered from rheumatoid arthritis and myeloma Rheumatoid arthritis developed five years earlier than the myeloma It is impossible to say whether malignant plasma cell clones had already developed by the end of the 1950 s As the predominance of malignant plasma cells steadily increased from 1964 onwards the rheumatoid arthritis showed steady inactivation At the same time the titre of rheumatoid factor in the blood decreased and finally this factor was no longer demonstrable It is obvious that when the one disease showed steady progression the activity of the other abated It may be assumed that the course of the arthritis was not altered by the cytostatic therapy given for three months The role of the steroid administration in the remission of the arthritis is not readily evaluated

## *Case 2*

A male economist born in 1907 In 1923 at the age of 16 he fell acutely ill with fever and symptoms from the joints and heart He was treated in hospital for half a year and gradually recovered although he had intermittent joint pain for the rest of his life During a journey to South America in 1931 he had an episode of malaria and dysentery After this he was in good health until the middle of the 1950 s when an exacerbation of the joint symptoms occurred In 1959 dyspnoea set in and the general condition deteriorated Definite signs of rheumatoid arthritis were then present joint pain swelling radiologically demonstrable destruction of the joints ESR ad 73 mm and a Waaler Rose titre of 240 During hospitalization in Sweden in 1962 a diagnosis of myeloma was based on elevation of the ESR bone marrow puncture showing 22 plasma cells and a pathological serum electrophoretic pattern with marked increase of beta globulins (Fig 2) Immunoelectrophoresis revealed an increase of IgG The myeloma was treated with melfalan for several short period X ray examination in 1963 revealed an infiltration in the apex of the left lung Active pulmonary tuberculosis was bacteriologically confirmed Tuberculostatic therapy was started In 1966 the arthritic symptoms abated to a marked degree and the clinical picture was entirely dominated by symptoms attributable to the myeloma In 1967 the following laboratory findings were made ESR over 120 mm Hb 8 0-9 0 g/100 ml Serum electrophoresis total proteins 8 g/100 ml 51 8 of which were beta globulins Immunoelectrophoresis gamma G proteinaemia On later examination (Odd Wager) using antikappa and antilambda immune sera the M component was found to belong to the kappa group It was thus monoclonal There was a marked decrease in normal immunoglobulins The bone marrow contained plasma cells to 70–60 X rays revealed no bone metastases The patient expired at the beginning of Jan 1968

*Autopsy findings*

The bone marrow showed the histological features typical of myeloma No skeletal myelomatous infiltrations were found Secondary amyloidosis was detected in the lymph nodes spleen and kidney but not in the synoviae The pulmonary infiltration was found to consist of histologically malignant tissue PAD carcinoma adenomatosum pulm s n.

### Comment

The first joint symptoms occurring at the ag of 16 should be attributed to an episode of rheumatic fever From the mid 1950s the diagnosis of rheumatoid arthritis was established The joint symptoms showed progression over many years A remarkable activity was noted during the first year after the diagnosis of multiple myeloma was made but four years later a remission of the rheumatoid arthritis occurred During that period no treatment with cytostatics was given

The remission of the joint disease may be the result of a depression of immune competent cells other than the myeloma clone The occurrence of tuberculosis and cancer of the lung are probably signs of depression of the immunological defence system too

## Case 3

A female industrial worker born in 1900 In 1941 six months after an episode of tonsillitis followed by tonsillectomy joint symptoms developed symmetrically in the basal toe joints and the metacarpophalangeal joints of the hands. The knee joints were affected a few years later A diagnosis of rheumatoid arthritis was made on the basis of the clinical picture and X rays showing typical rheumatoid destruction of the above mentioned joints The patient was given salicylate therapy

In 1949 she was treated for allergic dermatitis at the elbows and back of the knees The ESR was 5 mm.

In 1954 1955 and 1957 she had episodes of fever and pronounced joint symptoms. She was twice given gold therapy

In 1958 she had to give up working on account of the arthritis

At the end of 1959 and beginning of 1960 the general condition deteriorated and pain in the muscles of the upper arms and neck set in The joint symptoms were then subjectively somewhat better Anaemia was present The following laboratory values were noted Hb 6 5–9 5 g/100 ml erythrocytes ? 12–3 09 mill/mm ESR ad 148 mm, Waaler Rose 8 LE cells negative Moderate renal insufficiency with proteinuria ad 9 g/l was observed No Bence Jones protein was detected The total serum proteins were 12 3 g/100 ml and electrophoresis showed a monoclonal paraprotein peak between the beta and gamma fields constituting 54 of the total proteins (Fig. 3) Plasma cells were markedly predominant in bone marrow aspirate from the sternum X rays revealed typical myelomatous destruction of the diaphysis of the left humerus.

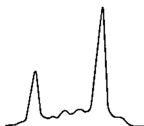


*Fig 3* Electrophoretic pattern of serum proteins in case 3

Urethan therapy was started The patient died of renal complications in June 1960 No post mortem examination was performed

### Comment

This patient had protracted and progressive rheumatoid arthritis She was completely disabled for two years before she died of myeloma The progression of the joint disease was retarded and the joint symptoms showed subjective improvement when the myeloma reached a severe stage

## Case 4

A housewife born in 1906 In 1952 pain and swelling of the joints developed These symptoms progressed for three years and there were episodes of fever and poor general condition In 1955 the patient was admitted to the Rheumatism Foundation Hospital The diagnosis of rheumatoid arthritis was made on the basis of a typical clinical picture radiologically demonstrable destruction of the finger joints, elevation of the ESR ad 85 mm and a Waaler Rose titre of 1 8 In the next year the serum electrophoretic pattern was pathological with increase of the beta globulin fraction ad 35 (Fig 4) In the autumn of 1957 the patient was treated for myeloma at the Second Department of Medicine Helsinki University Central Hospital The therapy consisted of urethanethylcarbamate 1 g × 2 ESR ad 130 mm, serum proteins ad 8 9 g/100 ml including 58 9 pathological beta globulins increase of plasma cells in the bone marrow and radiolucent myelomatous skeletal foci were noted. The patient was discharged in relatively good condition and free from joint symptoms In 1958 she had an acute inflammation of the eyes She died of myeloma in 1959 No post mortem examination was performed

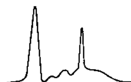


*Fig 4* Electrophoretic pattern of serum proteins in case 4

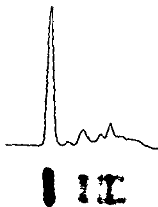



*Fig 5* Electrophoretic pattern of serum proteins in case 5

*Comment*

In this case too the initial diagnosis was rheumatoid arthritis a malignant plasma cell disease was diagnosed two years later According to the available data the joint disease still progressed after discovery of the myeloma When the extension of the latter disease had reached a severe degree th subjective joint symptoms abated It seems possible that the remission of the joint disease resulted from the urethan therapy given Amyloid degeneration cannot be ruled out.

*Case 5*

A male retired packing foreman, born in 1888 In 1947 joint symp oms developed symmetrically in the knee elbow and shoulder joints Three months later the proximal interphalangeal and metacarpophalangeal joints of the hands were affected. The patient was admitted to the Kive a Hospital, Helsinki Rheumatoid arthritis was diagnosed on the basis of the clinical picture X rays revealing destruction of the joints of the hands and elevation of the ESR ad 30 mm. The patient was treated by analgetics and blood transfusions. Probably as a result of the transfusions, hepatitis developed four months after his discharge from hospital

During the next twenty years the patient experienc d joint pains from time to time but he was able to work and did not require hospital treatment In 1967 diabetes was diagnosed later in the same year the ESR was elevated and electrophoresis showed a beta globulin peak. On admission to hospital at the beginning of 1968 the patient felt subjectively well, but he complained of intermittent increasing pain in the shoulder joints The tentative diagnosis of myeloma previously made on the basis of the high ESR and the electrophoretic finding (Fig. 5) was confirmed by immunoelectrophoresis, which showed indisputable IgA paraproteinaemia The same picture was obtained with monospecific anti IgA serum. The determinations with anti kappa and anti lambda serums reveals a M component with lambda type light chains In addition, large pathological plasma cells were found in abundance in the sternal marrow No Bence Jones protein was detected Tests for LE and rheumatoid factor were negative The patient is still alive

*Comment*

The patient suffered from subclinical rheumatoid arthritis when myeloma developed The prodromal phase of the myeloma was associated with exacerbation of the joint symptoms

*Case 6*

A farmer born in 1901 He has experienced intermittent, migrating pain in the joints for more than twenty years The main therapy has consisted of salicylates, which have proved ineffective In 1962 and 1964 he received cortisone therapy of short duration He was able to work and in fairly good condition until the autumn of 1967 when he fell acutely ill with rheumatoid arthritis.

In the spring of 1968 the following laboratory values were obtained ESR 123 mm, Hb 11 0 Waaler Rose 2000 latex fixation + + + anti nuclear antibodies negative LE phenomenon negative X rays showed typical destruction in the small joints of the extremities. Furthermore the following findings are noteworthy serum total proteins were 8 6 g/100 ml and electrophoresis showed a distinct peak between the beta and gamma globulins (Fig. 6) On immunoelectrophoresis an increase of IgG and IgA was noted. No M component was noted at electrophoresis in cellulose acetate The tests with anti lambda and anti kappa serums were normal. Ultracentrifugation of serum protein revealed a marked increase of IgG The bone marrow showed a marked rise in the number of plasma cells The shape of the ce ls varied, and numerous cells had two or three nuclei. Myeloma is suspected on the basis of the bone marrow finding, but X rays show no definite foci of myeloma The patient is being treated with dexamethasone 1 mg a day No cytostatics have been given.

*Comment*

The patient s joint disease is consistent with the diagnostic criteria of rheumatoid arthritis Marked plasma cell proliferation has b en observed, but no definitely malignant cells or cellular products The determinations of the immunoglobulins are still within normal range No M component has been observed This patient is a typical

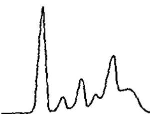


*Fig 6* Electrophoretic pattern of serum proteins in case 6.

exampl of a rheumatic with strongly stimulated but benign plasma cell clones

## DISCUSSION

A combination of rh umato d arthritis and multi ple myeloma in the same patient is possible The incidence of mye'oma in rheumatoid arthritis can not however be estimated on th basis of this study Clinical improvement of the joint symp toms was noted during progression of the plasma cell dyscrasia In one case the titre of circulating rheumatoid factor obviously decreased Dilution of the rheumatoid factor by increasing M compo nents probably contributed to the drop in titre A general depression of the antibody production after the development of myeloma was in one case typically reflected by the occurrence of a complicating tuberculous infection and a malig nant tumour

Plasma cell dyscrasia is used a synonym to Waldenstrom s term monoclonal gammopathy and paraimmunoglobulinopathy Multiple mye loma Waldenstrom s macroglobulinaemia amyloi dosis and heavy chain disease are today consider ed as belonging to this group of diseases More over a monoclonal gammopathy occurs the course of which is benign (19) Cryoglobulinaemia too is regarded as a manifestation of pathological plasma cell function

In Fig 7 an attempt has been made to cor relate the above mentioned plasma cell diseases to rheumatoid arthritis In addition Sjogren s syndrome or keratoconjunctivitis sicca and malignant lymphoma have been included

Amyloid degeneration is a serious complica tion in rheumatic cases In a manner that is so far unknown rheumatoid arthritis predisposes to amyloid deposition (9 10) Amyloidosis too often o curs as a complication of multiple myeloma In 1957 Davis et al (4) cited six cases of primary rheumatoid arthritis and/or multiple myeloma They added two cases of their own Some of these eight patients showed amyloid deposition others not Some of them exhibited indubitable symptoms of rheumatoid arthritis years before a diagnosis of myeloma was made The authors however considered the condition to be pseudo-rheumatoid arthritis Galli and Chiti (6) regarded plasmocytoma as secondary to rheumatoid arthri tis They published only one case At the begin ning of the 1960 s too patients with multiple

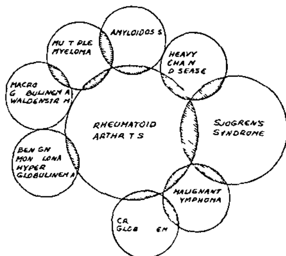



*Fig 7* Known clinical combinat on of rheumatoid arthri tis Sjogren s syndrome and plasma cell dyscrasia

myeloma presenting as rheumatoid arthritis were described Amyloid deposits were usually found in the joints (7 8) Hamilton and Bywaters (8) examined 46 patients with myelomatosis Joint symptoms sometimes developed before and some times after multiple myeloma was detected These authors posed the question whether rheumatoid arthritis which irritates the immune system some times develops into myeloma Since it has been proved that multiple myeloma may be asympto-matic for twenty years the relationship between this disease and rheumatoid arthritis and their or der of development are not readily assessed Hence there seems to be reason for a closer study of the relationship between rheumatoid arthritis and other pathological plasma cell reactions

In 1964 heavy chain disease (5 15) was added to the number of known plasma cell dyscrasias Among the ten cases so far described two are of interest in this connection At the Congress of Hematology in New York in 1968 Zawadzki et al (20) described a patient suffering from what they considered to be rheumatoid arthritis later heavy chain disease developed The joint disease had been present for nine years before the plasma cell dyscrasia was detected Wager et al (17) have reported a case of heavy chain disease con comitant with Sjogren s syndrome The point of time when the plasma cell dyscrasia developed in this patient cannot be established

Waldenstrom s macroglobulinaemia (18) has not been reported in rheumatics but this condi

tion occurs in association with myelomatosis (1)

Meltzer and Franklin (12) studied 29 patients with cryoglobulinaemia. Twelve of these had positive rheumatoid factor tests, and two showed, in addition malignant disease of the immunological system. The remaining patients were considered to have some systemic disease of the connective tissue One young patient with rheumatoid arthritis exhibited cryoglobulins consisting of IgG polymers with rheumatoid factor activity (13)

It seems safe to state that there is a definite positive correlation between rheumatic diseases and reticulosis (11) Malignant lymphoma occurs in association with rheumatic diseases (3) and Sjögren's syndrome (16) Talal and Bunim (16) reported a case of rheumatoid arthritis, Sjögren's syndrome and malignant lymphoma.

The reactive capacity of the plasma cells is diverse. As compared to cells producing albumins and globulins which do not participate in the immunological defence system, the immunologically competent cells show a wide range of degenerative possibilities (19) It is impossible to demonstrate a causal relationship in all of the combinations described. Protracted irritation of the immunological system, such as is certainly exerted by rheumatoid arthritis, may probably lead to reactions in the immunologically competent cells. Our cases were considered as primary rheumatoid arthritis with secondary malignization of the plasma cells in the form of multiple myeloma. The small number of cases does not allow of any definite conclusions regarding a positive correlation between rheumatoid arthritis and myeloma. However the cases reported in the literature lend support to the view that a causal relationship exists. The weak point in our chain of evidence lies in the fact that the synovia of all patients was not investigated for amyloid Amyloid deposition in the joints seems to give the clinical symptoms of rheumatoid arthritis.

When monoclonal M components become predominant in immune antibody synthesis, other antibodies and rheumatoid factor disappear In our cases clinical remission of the joint symptoms occurred. This would probably have been impossible if the joint symptoms had been due to amyloid deposition. A decrease in circulating rheumatoid factor has also been observed in a case of Sjögren's syndrome with malignant lymphoma (16)

## REFERENCES


1 Adner P L, Wallenius, G & Werner L. Macroglobulins in myelomatosis. Acta med. scand. 168 431, 1960
2 Bloch, K. J & Bunim, J J. Sjögren's syndrome and its relation to connective tissue diseases. J chron. Dis. 16 915 1963
3 Cammarata, R. J., Rodnan, G P & Jensen, W N. Systemic rheumatic disease and malignant lymphoma. Arch. intern. Med. 111 330 1963
4 Davis, Jr., J S., Weber F C & Bartfeld, H. Conditions involving the hemopoietic system resulting in a pseudorheumatoid arthritis, similarity of multiple myeloma and rheumatoid arthritis. Ann. intern. Med. 47 10 1957
5 Franklin, E. C., Lowenstein, J., Bigelow B. & Meltzer M. Heavy chain disease—a new disorder of serum gamma globulins. Amer J Med. 37 332, 1964
6 Gaul, T A. Chin, E. Rheumatoid arthritis and plasmacytomatosis. Ann. rheum. Dis. 14 2 1, 19 5
7 Goldberg, A., Brodsky I & McCarty D. Multiple myeloma with paramyloidosis presenting as rheumatoid disease. Amer J Med. 37 653 1964
8 Hamilton, E. B. D & Bywaters, E. G L. Joint symptoms in myelomatosis and similar conditions. Ann. rheum. Dis. 20 3 3 1961
9 Kindback, B & Wegelius, O. Secondary amyloidosis. A study of clinical and pathological findings. Acta med. scand. 180 737 1966.
10 Laine, V., Vainio K & Ruama, V. Occurrence of amyloid in rheumatoid arthritis. Acta rheum. scand. 1 43 1955
11 Lea, A. J. An association between the rheumatic diseases and the reticuloses. Ann. rheum. Dis. 23 480 1964
12 Meltzer M. & Franklin, E. C. Cryoglobulinemia. A study of twenty-nine patients. Amer J Med. 40 828 1966.
13 Meltzer M., Franklin, E. C., Elias, K. McCluskey R. T & Cooper N. Cryoglobulinemia. A clinical and laboratory study. Amer J Med. 40 837 1966.
14 Osserman, E. F & Fahey J L. Combined and chronic-plasma cell dyscrasias. Amer J Med. 44 256, 1 68
15 Osserman, E. F & Takatsuki, K. Clinical and immunochemical studies of four cases of Heavy chain disease. Amer J Med. 37 357 1964
16. Talal, N & Bunim, J J. The development of malignant lymphoma in the course of Sjögren's syndrome. Amer J Med. 36 529 1964
17 Wager O., Rasanen J A., Lindeberg, L & Makela V. Two cases of IgG heavy-chain disease. Acta path. microbiol. scand. In press.
18 Waldenström, J. Incipient myelomatosis or "essential" hyperglobulinemia with fibrinogenopenia—a new syndrome. Acta med. scand. 117 216, 1944
19 Waldenström, J. The Harvey Lectures, p. 31 Academic Press, New York 1960-1961
20 Zawadzki, Z. A., Benedek, T G., Ein D & Easton J M. Rheumatoid arthritis terminating in heavy chain disease. Proc XIIth Congr Int. Haematol, abstract p. 133 New York 1968

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# ANOREXIA NERVOSA SECONDARY ALDOSTERONISM AND ANGIOPATHY

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*Abstract* A female patient 29 years old is presented She exhibited an abnormal personality leading to anorexia nervosa diarrhoea of the ulcerative colitis type surreptitious vomiting severe hypokalaemia and secondary hyperaldosteronism. The occurrence of severe arterial and arteriolar thickening in conjunction with normal blood pressure and high blood renin activity is stressed

Recently attention has been paid to the occurrence of arteriolar and arterial thickening in various non hypertensive states associated with secondary aldosteronism (3 12 13) There are an increasing number of reports of psychologically disturbed patients in whom vomiting and abuse of laxatives have led to secondary aldosteronism (5 14 15) In some of the cases the juxtaglomerular complex has been hyperplastic and the blood renin activity high

This is a report of a patient exhibiting anorexia nervosa excessive vomiting diarrhoea of the ulcerative colitis type severe hypokalaemia and secondary hyperaldosteronism The occurrence of severe arterial and arteriolar thickening in relation to normal blood pressure and high blood renin activity is the main indication of this report

## CASE REPORT

The patient, a female cook, was the sixth of eight siblings and grew up in a foster home Somatic development and previous health were normal In 1960 at the age of 20 years, the patient first had abdominal symptoms in the form of pain and temporary diarrhoea She experienced some muscular weakness at that time

In August 1964 at the age of 24 the patient was admitted to another hospital because of severe diarrhoea and extreme exhaustion. Bloody diarrhoea occurred and the colon showed changes typical of ulcerative colitis both radiologically and by endoscopy The patient was therefore treated with Salazopyrin® and prednisone lavage Gradual improvement took place and she was discharged four weeks later

In October 1964 a new admission was precipitated by hypokalaemia muscular weakness and extreme fatigue Serum potassium was 1 2 mEq l There was usually a slight metabolic alkalosis. Blood pressure and haemoglobin levels were normal

During the following three years the patient attended an outpatient clinic, being admitted to the ward on three occasions The clinical picture was dominated by hypokalaemia hyponatraemia hypochloraemia and metabolic alkalosis. The blood pressure was always normal The urinary output was 000–3000 ml day and signs of impairment of renal function were seen (creatinine clearance 50 ml/min) Since the first admission amenorrhoea had developed. Study of the old files shows that frequent vomiting and occasional diarrhoea had been recorded by nurses. The patient and her doctors attributed no significance to these symptoms at that time

In October 1967 at the age of 29 years the patient was admitted to the renal ward She was cachectic The pigmentation of the skin was increased that of the mucosal membranes was normal In spite of wasting pubic hair and breasts were normal (Fig 1)

Hypokalaemia hyponatraemia hypochloraemia and metabolic alkalosis were present (Table I) The blood pressure was 105 80 mm Hg

The patient had continuous diarrhoea. The stool volume measured up to 1300 ml/day No blood was detected in the stools The Shilling test, FIGLU excretion serum vitamin $B_{12}$ and serum folic acid were normal The vitamin A tolerance test was normal Faecal fat content was normal The glucose galactose tolerance test showed a normal increase of serum glucose while the lactose tolerance test failed to increase serum glucose Jejunal aspiration biopsy revealed a normal mucosa with normal villi Estimation of disaccharidase activity of the jejunal biopsy tissue showed selective lactase deficiency Sigmoidoscopy and colography showed no actual changes of colitis ulcerosa and the biopsy taken from the sigmoid was normal

Hypokalaemia could not be corrected by substitution with more than 100 mEq day Balance studies, performed on two occasions three days and twenty two days respectively (Fig ) showed that the intake of electro-

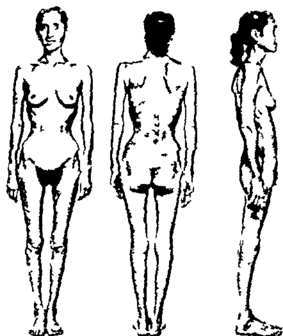


*Fig 1* The patient in October 1967

lytes was much greater than the total output that could be measured from the stools urine and occasional vomit This indicated that surreptitious vomiting must have occurred and the patient later admitted this The balance tudy also showed that the faecal loss of electrolytes was reater than the urinary loss Urinary potassium/sodium aried between 2 and 5 indicating hyperaldosteronism.

Serum creatinine was 1 0 mg/100 ml creatinine clear ance 60 ml/min excretion of phenolsulphonphthalein measured 44 /2 hours and isosthenuria was present Renal biopsy showed hyperplasia of the juxtaglomerular complex, important narrowing of medium-sized and small arteries and arterioles focal interstitial fibro is and hya linization of glomeruli in areas corresponding to the vascular alterations (Figs 3 4)

Aldosteronuria was measured twice (Table I) Plasma renin activity was measured three times according to th method of Boucher et al (2) Shortly after the first admission plasma renin activity was 490 ng/10 ml/3 h (control below 100 ng/10 ml/3 h) One year later it measured 233 ng/10 ml/3 h on a normal sodium diet and 309 ng/10 ml/3 h after a three-day sodium load of 2 0 mEq/die

Total body exchangeable potassium was measured twice using $K^{42}$ Total body exchangeable sodium was measured once using $Na^{24}$ (Table I)

Exogenous angiotensin elicited only a slight response in blood pressure during the angiotensin infusion test

The blood volume was measured several times (Table 1)

In addition to the vascular alterations seen in the renal biopsy angiopathy occurred in other parts of the arterial system. Biopsy of the quadriceps muscle showed thickened arteries of medium size and arterioles with hyperplastic walls (Fig 5) During the follow up period of two years

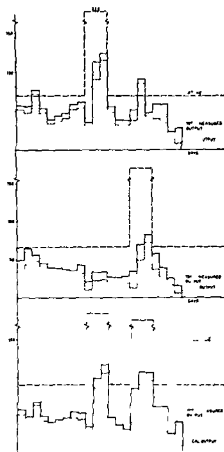


*Fig 2* Electrolyte balance study performed in November 1968

the patient experienced progressively increasing pain in her right foot After a period of evere intermittent claudication she was admitted because of threatening gangrene of the right great toe Angiography showed complete occlusion of the popliteal artery on the right side and extensive alterations of the femoral arteries and several branches on both sides (Fig 6)



*Fig 3* Hyperplasia of the juxtaglomerular complex. H E. × 180



*Fig 4* Small renal artery with thickened wall. H-E, ×180

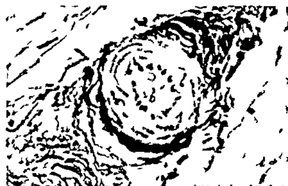


*Fig 5* Small artery with thickened media. Biopsy of the quadriceps muscle H-E ×180

Psychiatric consultation was performed. The behaviour of the patient, the anorexia, the surreptitious vomiting and the previous ulcerative colitis were easily explained according to the psychiatrist by abnormal personality resulting from the patient s exceptional early environment.

## DISCUSSION

A schematic representation of the suggested pathogenesis of the syndrome is given in Fig 7

The findings indicated that the patient was suffering from severe loss of potassium sodium and chloride The loss was due to severe chronic diarrho a, surreptitious vomiting and anorexia which later on appeared to be a dominating feature The patient also had lactase deficiency but a lactose free diet had only a transient effect on the volume and consistency of the stools The diarrhoea was therefore thought to be mainly due to the previous ulcerative colitis

The constant and longstanding loss of electrolytes had led to depletion of at least potassium and sodium and also to hypokalaemia and hyponatraemia During the early phases of the disease process hypovolaemia was a very probable component Hyponatraemia with or without concomitant hypovolaemia was responsible for the hyperreninism and hyperaldosteronism Exaggerated potassium loss resulted as well as sodium retention which led to partial reconstitution of the blood volume

Angiotensin activity was not measured in this case Hyperplasia of the juxtaglomerular complex high blood renin activity increased aldosteronuria and poor response to exogenous angiotensin are

Table I *Clinical and laboratory data*

| | Oct 67 | Feb 68 | Sept 68 | Jan 69 |
|---|---|---|---|---|
| Age (y ) | 29 | | | |
| He ght (cm) | 167 | | | |
| We ght (kg) | 38 (60) | | | |
| Plasma | | | | |
| Potassium (mEq/l) | 2 2 | 2 7 | 2 6 | 2 7 |
| Sod um (mEq l) | 129 | 133 | 131 | 136 |
| Chloride (mEq/l) | 93 | 97 | 86 | 95 |
| pH | 7 44 | 7 36 | 7 47 | 7 40 |
| Base excess (mEq l) | +4 8 | −1 3 | −13 1 | −0 6 |
| Creatinine (mg/100 ml) | 1 0 | 1 1 | 1 1 | 0 9 |
| Haematocr t ( ) | 44 | 30 | 40 | 38 |
| Creat nine clearance (ml/min) | 60 | 69 | 46 | 37 |
| Blood volume (l) | 3 0–3 2 (2 75) | | | |
| (ml kg) | 85 8–88 8 (76 5) | | | |
| Exchangeable body sodium (mEq) | 1470 (2340) | | | |
| Exchangeable body potassium (mEq) | 16 0–1460 (2400) | | | |
| Aldosteronur a (μg/die) | *30–50* | | | |
| B ood pressure (mm Hg) | 115/85–80 60 | | | |

Theoretical normal in parentheses



*Fig 6* Arteriography of femoral arteries performed in January 1969 *Note the complete occlusion of* right popliteal artery and the narrowings and ectasias of branches on both sides

evidence of an active renin angiotensin aldosterone system These features when accompanied by normal blood pressure are characteristic of the Bartter syndrome (1) and many clinical states involving secondary aldosteronism due to hypovolaemia and/or hyponatraemia (5 12 13 15)

Arterial and arteriolar thickening in spite of normal blood pressure has been reported in some of these states namely familial chloride diarrhoea (12) nephrotic syndrome (13) Bartter s syndrome (3) chronic abuse of laxatives (5) In the present case the vascular lesions are prominent and have become the most important symptom

In animal experiments severe arterial changes ve developed after administration of crude renin ts (11) In rats angiotensin infusion causes ension and arterial and arteriolar spasms and dilations (8 9) Even if hypertension is inhibited by dihydralazin vascular spasms and dilations occur when angiotensin is infused (9) Angiotensin has been shown to produce at least intrarenal lesions when given to rats over a lengthy period (10) In man angiotensin is a strong vasoconstrictor (7)

It is thus possible that the high renin angiotensin activity by causing longstanding vasoconstriction has given rise to arterial and arteriolar thickening especially in the kidney of this patient The unresponsiveness to exogenous angiotensin is compatible with high endogenous renin activity (4) and potassium depletion (6) The normal blood volume and high renin activity in spite of a sodium load suggest relative autonomy of renin release possibly due to the vascular changes in the kidney

In many cases of secondary aldosteronism a low effective blood volume makes the absence of hypertension understandable In the present case the blood volume was within normal limits and still the blood pressure was never elevated It is probable that there exists a relative vascular unresponsiveness to angiotensin due to tachyphylaxis i e the renin angiotensin aldosterone system is set at a higher level and only extremely high angiotensin activity is capable of increasing the systemic blood pressure

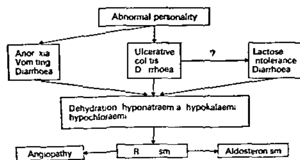



*Fig 7* Schematic representation of the suggested pathogen sis.

## REFERENCES


1 Bartter F C Pronove P Gill J R & MacCardle, R C Hyperplasia of the juxtaglomerular complex with hyperaldosteronism and hypokalaemic alkalosis a new syndrome Amer J Med 33 811 196‿

2 Boucher R Veyrat, R de Champlain J & Genest, J New procedures for measurement of human plasma angiotensin and renin activity levels Canad med. Ass J 90 194 1964

3 Brackett N C Koppel M., Randall R E & Nixon, W P Hyperplasia of the juxtaglomerular complex with secondary aldosteronism without hypertension (Bartter s syndrome) Amer J Med 44 803 1968

4 Catanzaro F Bourgoignie J Senrat P & Perry M H Angiotensin infusion tes Correlation with renin activity in peripheral venous blood Arch intern Med 122 10 1968

5 DeGraeff J & Schuurs M A M Severe potassium depletion caused by the abuse of laxatives One patient followed for 8 years. Acta med scand 166 407 1960

6. Fisher E. R. & Funckes, A J Effects of potassium deficiency on experimental renovascular hypertension. Lab Invest. 16 539 1967
7 Genest J Angiotensin aldosterone and human arterial hypertension Canad med Ass. J 84 403 1961
8 Giese J Acute hypertensive vascular disease I. Relation between blood pressure changes and vascular lesions in different forms of acute hypertension Acta path microbiol scand 62 481 1964
9 — Acute hypertensive vascular disease II. Studies on vascular reaction patterns and permeability changes by means of vital microscopy and colloid tracer technique Acta path microbiol scand 6 497 1964
10 Katz, Y J Patek, P R. & Bernick, S Effect of angiotensin on juxtaglomerular cells and vessels of the kidney Circulat. Res. 11 955 1962
11 Masson, G M C Kashii, C Panisset, J-C., Yagi S & Page I H Production of hypertension and vascular disease by kidney extracts. Circulat. Res. 14 150 1964
12 Pasternack, A. & Perheentupa J Hypertensive angiopathy in familial chloride diarrhoea Lancet 2 1047 1966
13 Pasternack A. & Tallqvist C. Non hypertensive angiopathy Renal biopsy study of the nephrotic syndrome Acta med scand 185 57 1969
14 Wallace M Richards P Chesser E. & Wrong, O Persistent alkalosis and hypokalaemia caused by surreptitious vomiting Quart J Med 38 577 1968
15 Wolff H P Kruck F Brown J J Lever A. F Vecsei P Roscher S Dusterdieck, G O & Robertson, J I. S Psychiatric disturbance leading to potassium depletion sodium depletion raised plasma renin concentration, and secondary hyperaldosteronism. Lancet 1 257 1968

# Congress Announcements

*Departement de Nephrologie Hopital Necker* (Jean Hamburger Jean Crosnier et Jean Louis Funck Brentano) *Cours de Perfectionnement sur la Nephrologie* les samedi 2 dimanche 3 et lundi 4 mai 1970

Il est recommande de s inscrire assez a l avance le nombre des participants etant limite Pour tous renseignements s adresser au secretariat du Professeur J Crosnier Hopital Necker 149 rue de Sevres Paris 15e

*The Tenth International Cancer Congress* will be held in Houston Texas May 22 to 29 1970

*Secretariat* Tenth International Cancer Congress P O Box 20465 Astrodome Station Houston Texas 77025 USA

*The Seventh Congress of European Dialysis and Transplant Association* will be held in Barcelona June 25 to 27 1970 The congress includes a scientific and commercial exhibition

*Information* from the Secretary Instituto Policlinico Platon 21 Barcelona Spain

*The Seventh Congress of the International Diabetes Fed ration* will be held in Buenos Aires Argentina August 23 to 28 1970

*Honorary Presidents* Charles H Best Canada and Bernardo A Houssay Argentina

*President of the Executive Committee* Dr Virgilio G Foglia Address VII Congreso Federacion Internacional Diabetes Paraguay 2155 7° Piso Buenos Aires Argentina

*An IAEA Symposium Dynamic Studies with Radioisotopes in Clinical Medicine and Research* will be held in Rotterdam the Netherlands August 31 to September 4 1970

*Organi ers* The International Atomic Energy Agency Karntnerring 11–13 1010 Vienna Austria

*Scientific Secretaries* Dr T Nagai and Dr E H Belcher Medical Applications Section

The Symposium will be concerned with all applications of radioisotopes in clinical medicine and research which involve measurements of the temporal patterns of uptake metabolism clearance or excretion of administered radioactive materials Topics to be covered include cardiac gastrointestinal hepatic pulmonary renal and thyroid function studies regional blood flow studies calcium copper iron protein and vitamin $B_{12}$ turnover studies and studies of red cell destruction The Symposium will give emphasis to new instruments techniques and methods of data analysis Studies based on scintigraphic techniques will be excluded except in so far as they are concerned with dynamic situations

Further information and forms to accompany abstracts of papers intended for presentation at the Symposium may be obtained from national authorities for atomic energy matters Abstracts must be submitted through these authorities so as to reach the International Atomic Energy Agency before April 20 1970

*The XI International Congress of Internal Medicine* will be held in New Delhi October 25 to 30 1970 President Dr R Viswanathan Secretary Dr H Vaishnava

Applications for taking part in the congress should be sent to the Secretariat before March 15 Address V P Chest Institute Delhi University Delhi India

Acta med scand Vol 187 pp 145-151 1970

# SOFT TISSUE CALCIFICATION IN HYPERPARATHYROIDISM

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*Abstract* Observations of soft tissue calcifications in a series of 71 cases of primary hyperparathyroidism are presented together with a review of the literature Four cases with unusual and illustrative features are presented in detail. From the localization, the appearance and the frequency the soft tissue calcifications in primary hyper parathyroidism can be divided into four groups. Soft tissue calcifications of diagnostic significance essentially nephrocalcinosis and peri and intraarticular calcification do not seem to occur in more than 5–10% of all cases with primary hyperparathyroidism. The phenomenon seems to be much more common in secondary hyperpara thyroidism. This is most probably due to the impaired kidney function usually found in secondary hyperpara thyroidism

Metastatic soft tissue calcification has been a well known phenomenon since it was first described in 1855 by Virchov (47) Extensive reviews of the literature and analyses of cases have been reported by Askanazy 1901 (6) Mulligan 1946 (32) and Selye 1962 (41) Quite early the con nection between metastatic soft tissue calcification and skeletal and renal disease was pointed out However it was not until 1923 that widespread soft tissue calcifications were described in a proved case of hyperparathyroidism The case published by Dawson and Struthers (12) showed widespread calcareous deposits not only in the excretion or gans—kidneys lungs and intestines—but also in th myocardium the liver the spleen the stomach the pituitary gland the pineal gland lymph nodes small and middle sized arteries and skeletal mus cles

Is soft tissue calcification a common finding in hyperparathyroidism? It is difficult to find the answer in the textbooks and reviews During later years the question has usually met with only a slight interest An exception is perhaps nephro-calcinosis but even in the case of this phenome non frequency figures vary considerably There are also other important questions Are there dif ferences between primary and secondary hyper parathyroidism are there special sites of predilec tion of the calcifications are there special factors favouring the metastatic calcium precipitation?

The present paper is a report of the findings in a series of 71 consecutive cases of primary hyper parathyroidism seen at the University Clinic 1958–1967 together with a review of the litera ture Four cases with unusual and illustrative features are presented in detail

## MATERIAL

From September 1958 to March 1967 71 cases of primary hyperparathyroidism have been nursed at the Medical Clinic Twenty of them were men and 51 women The age of the patients varied from 15 to 79 years Only seven patients were below 40 and 9 over 60 years old At operation or autopsy adenomata were found in 60 cases hyperplasia in six. In three cases the diagnosis was not histologically stated. Two patients refused opera tion

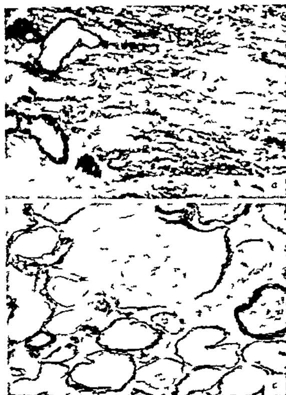
Examination of the cases with soft tissue calcification observed at the hospital during the same period revealed 21 cases of well documented hyperparathyroidism Table I gives a summary of the relevant data for 17 of the cases. In the remaining four cases there was calcification of the pineal gland only and the history and clinical picture were quite ordinary

In the search for soft tissue calcifications those found in arteries in patients over 50 were registered only when of remarkable extension

In addition to the 71 cases with proved hyperpara thyroidism there were three further cases of special in terest in this connection Those three are presented in detail together with one of the 21 in whom the histological findings were remarkable

Table I *Clinical data concerning the patients in the material reported*

| Case no | Age (y) | Sex | History | Calcifications | Serum calcium (m/Eq/l) | Serum phosph (mg/100 ml) | Urine calcium (mEq/24 h) | Creatinine (mg/100 ml) | Alkal phosph (Bessey-Lowry) | Histology |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 59 | ♀ | Kidney stones 15 y joint pains | Pineal gland calcanean spur | 5 3-6 0 | 1 8-2 6 | 8 5-11 9 | 1 2 | | Adenoma water clear and chief cells |
| 2 | 62 | ♀ | Joint and muscle pains 15 y osteit fibrosa | Tonsils + kidneys periarticularly left elbow leg arteries | 5 8-7 0 | 1 1-1 6 | 5 4-8 1 | 2 4 | 19 | Adenoma chief cells |
| 3 | 51 | ♂ | Osteitis fibrosa | Cartil disc left wrist menisci bilat | 7 1-7 7 | 1 6-2 3 | 18 8-31 2 | 1 1 | 1 8 | Prim hyperplasia water clear cells |
| 4 | 42 | ♂ | Peptic ulcer 21 y kidney stones 12 y hypercalcaemia | Lungs and pleura | 5 6 | 9 | 15 2-20 0 | 0 8 | 2 2 | Adenoma chief cells |
| 5 | 62 | ♀ | Kidney stones 5 y peptic ulcer 3 y hypercalcaemia | Aortic arch | 4 8-5 6 | 2 7-3 9 | 12 6-23 9 | 1 1 | 5 | Adenoma water clear and chief cells |
| 6 | 44 | ♀ | Kidney stones 7 y | Kidney choroidal plexus pineal gland | 5 3-6 5 | 2 2-2 4 | 13 5 | 1 1 | 1 7 | Adenoma (2) chief and transit cells |
| 7 | 60 | ♀ | Fatigue constipation | Pineal gland lungs | 5 0-5 8 | 2 1 | 16 8-22 2 | 0 7 | 3 4 | Adenoma chief cells |
| 8 | 61 | ♀ | Headache 3 y | Lungs pineal gland | 8 6 | 2 3 | 18 2-23 4 | 1 4 | 6 5 | Adenoma chief cells |
| 9 | 77 | ♀ | Peptic ulcer 20 y | Menisci of the knee uterine myofibroma | 6 8 | 1 8 | 2 5-4 4 | 0 9 | 1 2 | Adenoma water clear and chief cells |
| 10 | 40 | ♀ | Kidney stones 5 y hypercalcaemia 2 y | Falx cerebri + pineal gland | 5 4 | 3 0 | 32 | 0 7 | Normal | Adenoma chief cells |
| 11 | 40 | ♀ | Fatigue thirst vomit constip 2 y | Meningeoma + pineal gland | 5 3-6 3 | 2 3-3 0 | 16 6-19 5 | 0 6 | 0 9 | Adenoma chief cells |
| 12 | 66 | ♀ | Peptic ulcer 3 y recurrent urinary tract infections kidney stones 2 y | Lungs + kidneys | 6 0-6 5 | 1 8-2 4 | 10 0-15 5 | 0 8 | 2 5 | Adenoma chief and water clear cells |
| 13 | 71 | ♀ | Stomach ache 15 y kidney stones 10 y pancreat attacks 7 y diab 5 y | Lungs + aorta f moral and lienal arteries | 5 8-6 5 | 1 4-2 2 | 7 9-8 8 | 0 6 | 2 2 | Adenoma chief cells |
| 14 | 51 | ♀ | Kidney stones 1 y | Paratracheal glands + lungs + pineal gland | 5 4-5 7 | 2 1-3 5 | 14 8 16 2 | 0 8 | 9 6 | Adenoma chief cells |
| 15 | 67 | + | Increas ng fatigue and lassitude 3-4 mo | Most organs (see text case 4) | 8 5-10 2 | 3 7-5 0 | 3-25 | 1 0-1 9 | 4 4 | Adenoma chief cells |
| 16 | 62 | ♀ | Acute pancreat o te t f brosa? | Pleu a thyr aden m n sci and ap ules of th knee | 5 3-7 8 | 3 6-4 5 | 4 5-12 0 | 2 4 | 5 5 | Adenoma op |



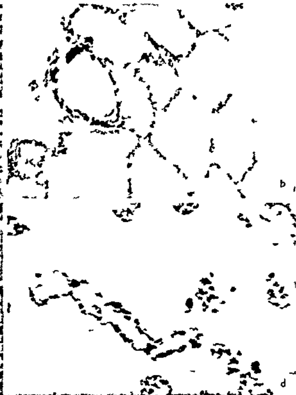




*Fig 1* (*a*) Case 2 Myocardium Extensive inter and intrafibrillar calc f cations (*b*) Case 4 Lung calc um deposit in alveolar s pta. (*c*) Case 2 Kidney Calcium deposit in basal membranes of g omerulus and tubules and in an arterial wall (*d*) Cas 4 Kidney Cal ium d pos.ts, mainly in tubular ep thelium. Stain, v Kossa.

## CASE REPORTS

*Case 1*

Male 73 years old, previously healthy Admitted on account of weight loss and pains in his stomach On examination there was an enlarged knotty liver but otherwise noth ng remarkable BP 130/80 Hb 73% NPN 1 0 mg% Ele ated transaminases and alkaline phosphatases ECG unspecific changes corresponding to the front wall of the left ventricle suspected old card ac infarction The state of the patient deteriorated and h died.

Autopsy showed a carcinoma of the pancreas with multiple liver metastases. Six enlarged parathyroid glands were found weighing together 13 g

M croscopic examination gave the diagnosis of hyper plasia with abundant water clear cells. Calcif cat ons were found n the tubular epithelium of the kidn ys, the myocardium the alveolar walls and the stomach wall.

*C s 2*

Female 38 without known renal disease in her family At the age of 32 hypertension ( 0/1 0) was discovered when she was examined for a health certificate The examination showed bilaterally cysti kidneys a slight hypertrophy of the left ventricle and proteinuria.

During the years she repeatedly had urinary tract infections and hematuria. At the age of 35 renal in suff c ency was definite

Calcium values in serum were normal in 1959 and 1962. Corre ponding inorgani phosphate alues were elevated, 8 0 and 11 7 mg% respectively Finally acute tracheobronchitis and circulatory insufficiency

The autopsy showed polycystic kidneys Three hyper plastic parathyroid glands were identified the dominant cell type of which was chief cells

Microscopy showed decalcifications in the kidneys af fecting mainly the basal membranes (F g. 1 *c*) the myocard um, the al eolar septa and the dura mater

*Case 3*

Female 72 years old. In 1949 progressive cutaneous sclerosis was d agnosed, engaging her hands and feet. In these areas as well as in other parts of the skin calcium-triphosphate crystals were demonstrated. During the years 1952–1962 X ray examinations revealed wide spread extensive calcifications in the lower extremities. Two small calcifications were also found in her tongue No roentgenological nephrocalcinosis was seen. The l boratory data showed a serum calcium l v l at the upper normal limit. Serum phosphate and calcium exc etion in the urine were usually normal. The phosphate clear ance was ele ated

Table II *The locali ation of soft tissue calcifications*

| | Primary hyperparathyroidism | | Secondary hyperpara thyroidism |
|---|---|---|---|
| | Earlier reported cases (*n*=65) | Own cases (*n*=21) | Earlier reported cases (*n*=14) |
| Nephrocalcinosis | 52 | 4 | 7 |
| Peri and intra articular tissue | 23 | 5 | 4 |
| Lungs hilar glands | 14 | 8 | 5 |
| Systemic arteries | 11 | 7 | 10 |
| Heart | 13 | 2 | 6 |
| Pancreas | 10 | 1 | — |
| Ventricle | 8 | 1 | 2 |
| Liver | 6 | — | — |
| Spleen | 4 | — | — |
| Dura | 2 | 3 | 1 |
| Pineal gland | 1 | 11 | — |
| Thyroid gland | 1 | 1 | — |

Three parathyroid glands were found on autopsy one of them of normal size the other slightly enlarged (8×4×4 mm) and the third clearly enlarged (10×5×5 mm) Sections from the two enlarged glands revealed a pronounced hyperplasia of oxyphil or chief cells In certain parts water clear cells were also seen Thus the chemical as well as the histological findings supported diagnosis of a moderate secondary hyperparathyroi dism (This case was previously described by Samuelsson and Werner (40))

*Case 4*

Female 67 years of age with moderate diabetes mellitus for 7 years During the last months rapidly increasing deterioration of the general condition On admission to the hospital she was semicomatous The clinical picture indicated hyperparathyroidism Calcium in serum 8 5–10 2 mEq phosphate 3 7–5 0 mg Despite an intensive therapy the patient died before operation could be carried out

At the autopsy two parathyroid glands were identified One gland contained a 35×10×10 mm adenoma weigh ing 1 9 g the other was normal The kidneys were of equal size and their combined weight was 380 g A calculus was found in the pelvis in the left kidney No calculi were seen in other parts of the urinary tract The heart weighed 490 g Moderate coronary sclerosis was present Patchy areas of extensive arteriosclerosis with occasional small ulcerations in the intima were seen in the aorta Patchy areas of fat necrosis were present in the pancreas Two encapsulated nodules of about 0 5 mm diameter were found in the cortex in the right adrenal gland

Microscopically the parathyroid adenoma was mainly composed of chief cells In the kidneys calcium deposits were located especially to the cortical zone and were found both in the tubular lumen and in the epithelium (Fig 1 c) Very little calcium was present in the inter stitial tissue glomeruli of the walls of blood vessels Calcium deposits were also found in the myocardium, the alveolar septa (Fig 1 a) in the muscular layers of the oesophagus and urinary bladder the intestinal wall, the necrotic areas in the pancreas in the arteries and in skeletal muscles

## DISCUSSION

In the literature we have found reports on 65 cases with primary hyperparathyroidism and soft tissue calcification In many of the cases the casuistic data are imperfectly recorded but the mean age of the patients seems to be around 50 years or slightly below A detailed report of clinical and biochemical data seems impossible to give as exact information is often missing Only in 14 patients have we found an index of the renal function This was reduced in 12 of the patients (NPN ⩾ 40 mg ) In the two cases with NPN below 40 mg % no nephrocalcinosis was reported On the other hand it is possible to give a more complete account of the distribution of the soft tissue calcifications in different organs The data are based on papers 2 4 5 7 8 10 12 13 15 16 17 18 19 20 22 25 26 27 29 31 33 34 35 43 46 48 The site and frequency of the abnormal calcifications are demonstrated in Table II As can be seen the most common sites of calcifications are the kidneys the lungs the systemic arteries peri and intraarticular tissue the pineal gland the heart and possibly the pancreas Less common are calcifications in the dura mater the gastric wall the liver and the spleen Definitely rare are calcifications in the skin the intestinal wall and the skeletal muscles

Thus to judge from previous reports the kid ney is the most common site of soft tissue cal cifications in primary hyperparathyroidism In a series of 138 cases of primary hyperparathyroi dism Hellstrom and Ivemark (20) report a nephro calcinosis frequency of 21 6 Similar findings were reported by Leman and Donatelli (27) In our material of 71 patients with primary hyper parathyroidism signs of nephrocalcinosis were found in only four patients i e in 6% This figure agrees well with most materials from the last decade (7 11 24 30 39 45)

The differences between the frequency figures are thus considerable One explanation could be that the number of kidney biopsies or autopsies was higher in the materials with high frequency of

nephrocalcinosis It has been shown that nephrocalcinosis can be demonstrated microscopically in many cases where the X ray findings have been negative As far as can be judged however no such differences are to be found

Another possibility is that the materials differ in composition i e the selection of patients is different Thus a material collected in surgical or urological departments could reasonably be expected to contain more patients with kidney stones and urinary tract disease than a material found in a department of general medicine As a matter of fact the series with a high percentage of nephrocalcinosis urologic cases and cases with *advanced kidney disease seem to dominate* Such cases are much less common in series with a low percentage of nephrocalcinosis We believe this to be the main explanation of the discrepancy Consequent y the low figures more truly reflect the real incidence of nephrocalcinosis in clinical hyperparathyroidism

The second most frequent site of calcification is peri or intraarticular tissues In our material they are just as common as nephrocalcinosis In the cases found in the literature they are much less commonly reported than is nephrocalcinosis This may be due partly to the overrepresentation of nephrocalcinosis in some series as mentioned above but partly also to the fact that nephrocalcinosis is usually of great clinical importance whereas the articular calcifications are clinically insignificant and thus might easily be overlooked

The localization appearance and frequency of the soft tissue calcifications in hyperparathyroidism make a division into four groups natural To the first group is referred nephrocalcinosis and peri and intraarticular calcifications These phenomena are seen much more often in cases with hyperparathyroidism than in the average hospital material in corresponding age groups

To the second group may be referred localized calcifications in the lungs the hilar glands and pleura, and calcifications of the pineal gland These types of calcifications occur often in hyperparathyroidism—as a matter of fact in our materials pineal gland calcification was the most common finding—but apparently they occur just as often in patients with normal parathyroid function

A third group is formed by the calcifications in systemic arteries the heart the pancreas and the dura Calcifications thus located occur fairly often in patients with normal parathyroid function but it is possible that they are more common and more severe in cases with hyperparathyroidism

To the fourth group finally may be referred calcifications located to the stomach wall the intestinal walls skeletal muscles the skin liver and interstitial calcifications e g in alveolar septa These types of calcification are a rare phenomenon apparently developing late in the course of he disease Besides they seem to be especially common in cases with clinically severe hyp rparathyroidism They appear practically always together with *widespread calcifications in other sites*

It has long been known that soft tissue calcification notwithstanding the primary cause has a predilection for certain organs and tissues It has been suggested that a local low pH might be the precipitating factor (41) Such a low pH can occur in organs such as the heart the lungs and the systemic arteries the arterial blood of which has a low content of carbon dioxide It can also occur in organs which excrete acid components such as the kidneys the stomach and the lungs Necrotizing tissue and hematomata will also commonly be calcified for reasons that are not as easily understood Furthermore tissues like the dura mater synovial and cartilaginal structures also seem to have a tendency to abnormal calcification This is possibly due to the close kinship between these structures and osteoid tissue It is apparent that the soft tissue calcifications of primary hyperparathyroidism in the main follow this general pattern There is thus nothing specific in the gross localization of the calcifications in hyperparathyroidism

Histologically the calcifications of groups 2 and 3 do not differ essentially from corresponding calcifications in individuals with normal parathyroid function The same is true for the peri and intraarticular calcifications The microscopic pictures of the nephrocalcinosis as well as the calcifications in group 4 often have an unusual appearance The calcium precipitation in nephrocalcinosis seems to have a special affinity for the basal membranes both of the glomerulus and tubulae (Fig 1 *a*) The calcifications e g in alveolar septa in the ventricular wall and striated muscles may also have a characteristic appearance (Fig. 1 *b*)

However the changes are not absolutely specific not even those of type 4 They may rarely be encountered in other states of disease such as vitamin D intoxication To judge from our own experience and from most recent reports there is no close correlation between soft tissue calcification and the clinical type of hyperparathyroidism nor is there any good correlation to the serum calcium levels It is reasonable to assume that hypercalcaemia must be a facilitating factor but obviously a high calcium level is not per se enough Of course it can be argued that the calcium figures are figures for total serum calcium and do not necessarily give information as to the amount of ionized calcium which might be the essential factor Our lack of knowledge on this point makes further discussion futile

In all cases with widespread and extensive soft tissue calcifications there has been a severe renal insufficiency with uraemia and a varying degree of phosphate retention This type of changes often seems to develop within a fairly short period In one case for example extensive calcifications appeared within a few months after the onset of renal insufficiency In these cases of extensive calcifications the most essential feature seems to be the phosphate retention For simple chemical reasons a rising serum phosphate level together with hypercalcaemia must result in calcium phosphate precipitation as the product (Ca) × ($PO_4$) will easily reach the level of maximum solubility of $Ca_3(PO_4)_2$

A number of cases with soft tissue calcification and secondary hyperparathyroidism have also been reported (1 3 7 9 21 23 28 36 37 38 42 44) The total number of cases was 14 the mean age 24 with a variation from $^1/_2$ year to 45 Eleven were men and three women The serum calcium values were normal or slightly elevated the serum phosphate values were all high All cases had a severely impaired renal function the NPH ranging from 125 to 364 mg per 100 ml The number of hyperplastic parathyroid glands varied On the average they were 3–4 The original diagnosis was chronic glomerulonephritis in eight cases chronic pyelonephritis in six The distribution of the soft tissue calcification is shown in Table II As will be seen the frequency pattern of soft tissue calcification in secondary hyperparathyroidism does not differ from that of primary

It is of course difficult to estimate the incidence of abnormal soft tissue calcification in the two types of hyperparathyroidism If those normally occurring in the lungs the hilar glands the pineal gland and in the arteries in elderly people are excluded the total incidence seems to be fairly low Soft tissue calcifications in primary hyperparathyroidism do not seem to appear in more than 5–10 % of the cases

It is still more difficult to estimate the true frequency of soft tissue calcifications in secondary hyperparathyroidism The scarcity of the well reported cases in the literature the relative rarity of cases in modern hospital material and the usually advanced state of kidney insufficiency at the time of diagnosis make a good estimation impossible However soft tissue calcification seems to be much more common in secondary hyperparathyroidism to judge from the literature and from our own experience it probably occurs in more than 50 %

## REFERENCES


1 Albright, F Baird P C Cope O & Bloomberg, E Amer J med Sci 187 49 1934
2 Alexander H B Pemberton H L Kepler E J & Broders A Amer J Surg. 55 157 1944
3 Andersen D H & Schlesinger E R Amer J Dis Child 63 102 1942
4 Anderson W A D Endocrinology 24 37 1939
5 Ask Upmark, E Acta med. scand. 76 128 1931
6 Askanazy M Verh dtsch Ges Path 3–4 72, 1901
7 Bogdonoff M D Woods A H White J E & Engel F L Amer J Med 21 583 1956
8 Bywaters E G L Dixon, A St J & Scott J T Ann. rheum Dis 22 171 1963
9 Castleman, B & Mallory T B New Engl J Med 214 320 1936
10 Cope O Surgery 16 273 1944
11 — Amer J Surg. 99 394 1960
12 Dawson J W & Struthers J W Edinb med J 30 421 1923
13 Ellis J T & Barr D P Amer J Path 27 383 1951
14 Engfeldt B & Lagergren, D Acta chir scand. 115 46 1958
15 Fontana A. Endocrinol e pat cosut 4 401 19 9
16 Gissel H & Bufe W Dtsch Z Chir 256 58 1942
17 Hand J R Surg Clin N Amer 13 1365 1933
18 Hanes F M Amer J med Sci 197 85 1939
19 Havard C H W Proc roy Soc Med 5? 1049 1959
20 Hellstrom, J & Ivemark, B I Acta chir scand. Suppl. 294 1962
21 Herbert, F K Miller H G & Richardson, G O J Path. Bact 53 161 1941

22. Hoffheinz, D Virchows Arch path Anat 256 705 1925
23. Hubbard R. S & Wentworth J A Proc. Soc exp *Biol (N Y) 18 307 1921*
24. Keating F R. Jr J Amer med Ass 178 547 1961
25. Khurgina P A Klin Med. 2 1735 1933
26. Laubman W Verh dtsch path Ges 27 9 1934
27. Lemann, J Jr & Donatelli A. A. Ann. intern. Med. 60 447 1964
28. Magnus, H A & Scott, R B J Path Bact 42 665 1936
29. Meyer O Frankfurt Z. Path 20 115 1917
30. Mieher W C. Jr Thibaudeau Y & Frame B Arch. intern Med. 107 361 1961
31. Mixter C. G Jr Keynes, M K. & Cope O New Engl J Med 66 265 1962
32. Mulligan, R. M Amer J Path 22 1 93 1946
33. Oliver W A Lancet 2 240 1939
34. Paul F Beitr path. Anat. 87 503 1931
35. Penecke P Zbl allg Path path Anat 37 535 19 6.
36. Pollack H & Siegal S J Mt Sinai Hosp 2 270 1936
37. Pons J A & Pappenheimer A. W Puerto Rico J publ Hlth 13 115 1938
38. Price N L. & Davie T B Brit J Surg 24 548 1937
39. Riddick, F A Jr & Reiss, B Ann intern Med 56 *183 1962.*
40. Samuelsson S M & Werner L Acta med scand *177 673* 1965
41. Selye H Calciphylaxis The University of Chicago Press, Chicago 1962.
42. Shelling D H & Remsen, D Bull Johns Hopk. Hosp 57 158 1935
43. Smith F B & Cooke R. T Lancet 2 650 1940
44. Smyth F S & Goldman L. Amer J Dis. Child. 48 596 1934
45. Steinbach, H L Gordan, G S Eisenberg, E. Crane J T Silverman, S & Goldman, L. Amer J Roentgenol. 86 329 1961
46. Turchi, J J., Flandreau R. H Forte A. L., Frenche G N & Ludwig, G D J Amer med. Ass 180 799 1962.
47. Virchov R. Virchows Arch path Anat. *8 103 1855*
48. Young, M O & Halpert, B Arch Path. 44 6 8 1947

# Congress Announcements

*The Fourth World Congress of Gastroenterology* will be held in Copenhagen July 12 to 18 1970

*Secretariat*

Before and after the congress Fourth World Congress of Gastroenterology c/o DIS Congress Service Skindergade 36 DK 1159 Copenhagen K Denmark

During the congress Bella-Centret Hvidkildevej 64 DK 2400 Copenhagen NV Denmark

*The Second World Congress on Ultrasonic Diagnostics in Medicine* will be held in Rotterdam June 4 to 8 1973

*President* Dr M de Vlieger

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# PLASMA LECITHIN CHOLESTEROL ACYLTRANSFERASE AND ERYTHROCYTE LIPIDS IN LIVER DISEASE

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*Abstract* Plasma lecithin-cholesterol cyltransferase (LCAT) activity was reduced to approximately one fourth of normal values in patients with chronic parenchymatous liver disease without marked jaundice (mean serum bilirubin ?0 mg/dl) A significant increase in red cell cholesterol was concomitantly demonstrated with a highly significant correlation between red cell cholesterol content and the concentration of plasma free cholesterol

In patients with marked obstructive jaundice (mean serum bilirubin 14 6 mg/dl) the plasma LCAT activity was even lower and in some hardly detectable This reduced activity could not be explained by LCAT inhibition in plasma. Erythrocyte cholesterol content was markedly increased up to twice the normal values A significant increase in erythrocyte lecithin and significant decreases in sphingomyelin and phosphatidyl ethanolamine were also found Erythrocyte cholesterol content was found to be normal in patients with hyperbetalipoproteinemia and marked elevation of plasma total cholesterol with normal plasma cholesterol esterification.

Glomset (9) has suggested that the plasma lecithin-cholesterol acyltransferase (LCAT) reaction is important in the cholesterol membrane homeostasis

In the study of patients with familial LCAT deficiency (7 8 13) we demonstrated abnormal lipid composition of the erythrocytes Cholesterol and lecithin contents were up to twice the normal whereas sphingomyelin and phosphatidyl ethanolamine were significantly reduced

LCAT is probably synthesized in the liver (9) and its activity may depend on liver function We have therefore studied this plasma enzyme activity in patients with different liver diseases and put particular emphasis on the relationship between the erythrocyte lipids the LCAT activity and the cholesterol esterification in their plasma

## MATERIAL AND METHODS

Ten patients with chronic parenchymatous liver disease chronic hepatitis or liver cirrhosis and six patients with obstructive jaundice of long duration were stud ed They were all inpatients at the Medical Department A of the University Hospital Oslo Diagnoses were established by means of routine function tests as presented in the tables needle biopsies of the liver X ray investigations and in some patients liver scanning All patients with obstructive jaund ce had their diagnosis establi hed at laparotomy

Blood samples for lip d studies were drawn from an antecubital vein in the morning after twelve hours fasting. No special diet was used on the previous day Anticoagulation of the blood was achieved with citrate dextrose solution (7) The sample was chilled and red cells immediately isolated by centrifugation at 4 C washed three times with cold 0 9 NaCl solution and resuspended in 0 9‰ NaCl to a hematocrite of 35-55% Al quots of the suspens on were pipetted off for automatic red cell counting and lipid extraction

*Extraction of lipids* from erythrocytes was performed according to procedure III of Ways and Hanahan (20) The lipids were separated by means of thin layer chromatography using s lica gel H (Merck) washed w th chloroform-methanol formic acid as described by Parker and Paterson (16) Chromatograms were developed with chloroform-methanol acetic acid-water (25 14 4 2 by vol.) according to the method of Skipski et al (19) Extraction and chromatographic separa on were in all cases completed on the day when the blood was drawn to prevent degradation The known major red cell phospholipids separated di tinctly w th th s solvent system (7)

Cholesterol determination was performed in the eluate of the solvent front area. It was evaporated under a stream of air at room temperature Cholesterol was determined by a ferric chloride method (4)

LCAT activ ty was determined as follows

Plasma was preincubated for four hours with labelled cholesterol n the presence of a dithiol wh ch inhib ted the LCAT The enzyme assay was started with the addition of excess mercaptoethanol The incubation continued

Table I*a* *Patients with chronic hepatitis/liver cirrhosis Clinical and hematological data*

| Patients | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| No | Sex | Age (y) | Diagnosis | Duration of symptoms | Therapy | *Hb* (g/100 ml) | *RBC* (mill µl) | *WBC* (µl) | Platelets (µl) |
| L 3 | ♂ | 21 | Cirrhosis hepatis<br>Colitis ulcerosa | 2 y<br>6 y | Prednisone<br>Busulphan | 80<br>(11 8) | 4 06 | 5 800 | 299 000 |
| L 4 | ♀ | 20 | Hepatitis chron. | 1½ y | None | 75<br>(11 8) | 4 28 | 2,900 | 88 000 |
| L 5 | ♀ | 21 | Hepatitis chron.<br>(lupoid) | 1 y | Prednisone<br>Azathioprine | 77<br>(11 5) | 3 88 | 8,500 | 202,000 |
| L 6 | ♂ | 21 | Hepatitis chron | 6 y | Splenectomy and<br>splenorenal shunt | 103<br>(15 2) | 5 03 | 5 700 | 342,000 |
| L 7 | ♀ | 62 | Cirrhosis hepatis | 8 y | Prednisone | 89<br>(13 2) | 4 40 | 5 100 | 105 000 |
| L 11 | ♀ | 54 | Cirrhosis hepatis | 2 mo | None | 81<br>(12.1) | 4 15 | 5,200 | 188 000 |
| L 12 | ♀ | 46 | Cirrhosis hepatis | 6 mo | Splenectomy and<br>splenorenal shunt | 84<br>(12 4) | 4 10 | 11 600 | 527,000 |
| L 13 | ♀ | 22 | Hepatitis chron | 9 y | Splenectomy and<br>splenorenal shunt | 81<br>(12 1) | 3 93 | 5,500 | 202,000 |
| L 14 | ♀ | 16 | Hepatitis chron.<br>(lupoid) | 2½ y | Prednisone<br>Azathioprine | 75 | 3 50 | 5 600 | 87 000 |
| L 16 | ♂ | 63 | Cirrhosis hepatis | 2 y | None | 94<br>(13 9) | 4 09 | 10 600 | 133 000 |

for one hour and the labelled cholesteryl ester was counted after lipid extraction and thin layer chromatography A detailed description of the method will be given elsewhere (15)

## RESULTS

*Chronic parenchymatous liver disease*

Table I*a* presents the clinical data, therapy and essential hematological status in the patients with chronic hepatitis/liver cirrhosis. The results of the liver function tests are shown in Table I*b* It is seen that the transaminase values are slightly increased in all patients (normal values SGOT < 18 U/l and SGPT < 20 U/l) Alkaline phosphatase (normal < 45 U/l) was increased in eight and bilirubin increased above 2 0 mg/dl in four patients None had marked jaundice Serum albumin was low in eight patients and serum gamma globulin increased in all

The results of the plasma lipid studies are presented in Table II It is seen that the total cholesterol was within normal range in nine patients.

Table I*b* *Patients with chronic hepatitis/liver cirrhosis Liver function tests*

| Pat no | SGOT (U/l) | SGPT (U/l) | Alk. phosph (U/l) | Bilirubin (mg/dl) | Thymol turbidity (U) | Normo-test (NT) (% of normal) | Thrombo-test (TT) (% of normal) | Serum Albumin (g/dl) | Serum γ-globulin (g/dl) |
|---|---|---|---|---|---|---|---|---|---|
| L 3 | 64 | 75 | 190 | 4 8 | 0 09 | 95 | 35 | 3 0 | 2.5 |
| L 4 | 120 | 60 | 100 | 2.3 | 0 28 | 38 | 30 | 3 2 | 2.6 |
| L 5 | 29 | 31 | 85 | 0.5 | 0 12 | 105 | 78 | 3 4 | 1 9 |
| L 6 | 73 | 99 | 78 | 1 5 | 0 13 | 78 | 74 | 3 7 | 2.6 |
| L 7 | 94 | 83 | 126 | 1 5 | 0 43 | 26 | 37 | 2.4 | 4 4 |
| L 11 | 50 | 37 | 170 | 2.0 | 0 40 | 100 | 100 | 2 9 | 2.5 |
| L 12 | 68 | 43 | 294 | 3.2 | 0 17 | 35 | 19 | 2 9 | 2.3 |
| L 13 | 36 | 19 | 42 | 1 7 | 0 23 | 38 | 44 | 2.0 | 2.1 |
| L 14 | 47 | 52 | 45 | 1 2 | 0 34 | 66 | 66 | 3 5 | 4 5 |
| L 16 | 34 | 36 | 100 | 2.1 | 0 15 | 56 | 48 | 2.7 | 3 5 |
| Mean | 61 | 53 | 123 | 2.0 | 0.23 | 63 | 53 | 2.9 | [illegible]9 |

| ESR (mm/h) | Reticulocytes (/1000 red cells) | Serum iron (µg/100 ml) | Transferrin (µg/100 ml) | Osmotic fragility |
|---|---|---|---|---|
| 71 | 4 | 61 | | Normal |
| 0 | 8 | 75 | 380 | Decreased |
| 40 | 7 | 150 | 300 | Normal |
| 20 | 8 | 152 | 350 | Decreased |
| 36 | | 145 | | Normal |
| 110 | 15 | 176 | 282 | Normal |
| 71 | 16 | 90 | 3 0 | Decreased |
| 10 | 19 | 105 | 142 | Normal |
| 4 | 15 | 92 | 370 | Normal |
| 63 | 14 | 200 | | Normal |

Mean cholesterol esterification in per cent was below normal $-64 \pm 7$ % compared to 75% ±3% in ten normals Plasma triglycerides were normal in all Total phospholipids were also within normal range The distribution of the phospholipid fraction however deviated from the normal pattern as lecithin was slightly increased and sphingomyelin and lysolecithin fractions were below normal

Lipoprotein electrophoresis showed a normal pattern in five increased betalipoproteins in three a broad beta band in one and a marked alphaband in one of the patients

LCAT activity in plasma was markedly reduced in all patients 0 60% ± 0 37% free cholesterol esterified per hour compared to 3 0% ± 0 56 in ten normal subjects

The results of the erythrocyte lipid studies are given in Table III The cholesterol content of the red cells was increased 1 44 ± 0 22 $10^{-10}$ mg cholesterol per cell compared to the normal 1 11 ±0 11 This difference from the normal is statistically highly significant ($p<0\ 001$) Only one of the patients (L 14) had erythrocyte cholesterol within the normal range

Fig 1 shows that a highly significant correlation ($p<0\ 001$) exists between the concentration of free cholesterol in plasma and erythrocyte cholesterol content

Total phospholipids of the erythrocytes did not deviate from normal However slight deviation from the normal pattern of the individual phospholipid fractions was seen with a small decrease in phosphatidyl ethanolamine and a slight increase in the lecithin content. Red cell phosphatidyl serine sphingomyelin and lysolecithin did not differ from the normal

*Obstructive jaundice*

Six patients with obstructive jaundice of more than one month s duration were studied Their clinical and hematological data are shown in Table IV *a* It is seen that a decrease in the red cell osmotic fragility was found in all of them As in the group of patients with chronic hepatitis /liver cirrhosis Coombs test was negative in all Their liver function tests demonstrated a slight increase in transaminase values a marked increase in alkaline phosphatase and a marked jaundice with mean bilirubin of 14 6 mg/dl They all had low serum albumin Increased gamma globulins were found only in half of them

The plasma lipid studies (Table V) revealed high total cholesterol with low esterification of average 26 % significantly lower than in the chronic hepatitis group Serum triglycerides were above normal in all Total plasma phospholipids were elevated Also in this group plasma lecithin was increased and lysolecithin low

Lipoprotein electrophoresis showed the normal pattern in one In the others alphalipoproteins could not be detected and the beta band was broad in four patients

Plasma LCAT activity is seen to be very low in all these patients

Erythrocyte lipid studies of the patiens with obstructive jaundice revealed a marked increase in red cell cholesterol content $-1\ 86 \pm 0\ 40$ $10^{-10}$ mg/cell This is significantly higher than in the hepatitis/cirrhosis group ($p<0\ 05$) The total phospholipids were slightly elevated but this increase above normal was not statistically significant ($p<0\ 1$) Lecithin was significantly higher than that seen in the normal ($p<0\ 001$) and phosphatidyl etholamine ($p<0\ 05$) and sphingomyelin ($p<0\ 05$) lower Phosphatidyl serine was normal

A group of five patients with primary hyper

Table II *Patients with chronic hepatitis/liver cirrhosis Plasma lipid values lipoprotein pattern and LCAT activity*

| | Plasma lipids | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cholesterol | | | Tri glycerides (mg/dl) | Phospholipids | | | | | | Lipoprotein electrophoresis | LCAT activity | |
| | | | | | | Per cent of total | | | | | | | |
| Pat no | Total (mg/dl) | Free (mg/dl) | Esterif ( ) | | Total (mg/dl) | P E | P S | L | S | L L | | ( F C est /h) | (mμmole/ ml/h) |
| L 3 | 246 | 93 | 62 | 52 | 223 | 2 7 | 0 3 | 85 7 | 9 4 | 1 9 | β lp normal Marked α band | 0 32 | 8 |
| L 4 | 198 | 80 | 60 | 46 | 161 | 2 3 | 0 2 | 84 7 | 11 3 | 1 6 | Normal | 0 70 | 14 |
| L 5 | 275 | 101 | 65 | 55 | 155 | 4 2 | 0 9 | 74 8 | 16 9 | 3 2 | β band somewhat broad | 0 53 | 14 |
| L 6 | 318 | 124 | 61 | 83 | 203 | 4 2 | 0 5 | 82 3 | 11 4 | 1 9 | Slight increase in β lp otherwise normal | 0 24 | 8 |
| L 7 | 223 | 47 | 79 | 68 | 159 | 3 2 | 0 8 | 81 3 | 12 1 | 2 6 | Normal | 0 63 | 8 |
| L 11 | 414 | 165 | 60 | 115 | 310 | 1 9 | 0 3 | 82 9 | 11 7 | 1 2 | Increased β lipo protein | 0 43 | 20 |
| L 12 | 344 | 116 | 66 | 112 | 248 | 3 0 | 1 4 | 82 7 | 10 4 | 2 5 | Increased β lipo protein | 0 95 | 29 |
| L 13 | 187 | 97 | 66 | 59 | 141 | 3 6 | 3 2 | 78 8 | 12 5 | 1 9 | Normal | 0 37 | 10 |
| L 14 | 120 | 47 | 61 | 31 | 123 | 2 8 | 1 5 | 82 8 | 11 11 | 1 8 | Normal | 1 47 | 11 |
| L 16 | 295 | 114 | 62 | 7[illegible] | 196 | 2 3 | 2 2 | 90 2 | 4 2 | 1 1 | Normal | 0 35 | 11 |
| Mean | 262 | 96 | 64 | 69 | 192 | 3 0 | 1 1 | 82 6 | 11 1 | 2 1 | | 0 60 | 13 3 |
| ± S D | 112 | 42 | 7 | 27 | 56 | 0 7 | 0 9 | 5 2 | 3 0 | 0 7 | | 0 37 | 6 |
| Normal range | 180–340 | | 70–80 | 10–140 | 150–300 | | | 68–78 | 1[illegible]–22 | 3 7–9 3 | | 3 07±0 56 | 56 8±11 3 |

*Abbreviations*
P E = phosphatidyl ethanolamine P S = phosphatidyl serine L = lecithin (phosphatidyl choline) S = sphingomyelin L L = lysolecithin F C = free cholesterol

Table III *Patients with chronic hepatitis/liver cirrhosis Erythrocyte lipids*

Abbreviations as in Table II

| Pat no | Cholesterol ($10^{-10}$ mg/cell) | Phospholipids Total ($10^{-12}$ mg/cell) | Per cent of total PE | PS | PI | L | S | LL |
|---|---|---|---|---|---|---|---|---|
| L 3 | 1 53 | 10 4 | 23 4 | 9 0 | 1 0 | 41 8 | 23 6 | 1 2 |
| L 4 | 1 41 | 9 0 | 22.3 | 14 0 | 1 0 | 34 8 | 26 8 | 1 1 |
| L 5 | 1 22 | 10 5 | 21 7 | 18 2 | 1 1 | 33 7 | 24 2 | 1 1 |
| L 6 | 1 50 | 11 3 | 22.4 | 20 4 | 1 3 | 30 0 | 24 1 | 1 8 |
| L 7 | 1 23 | 10 4 | 17 5 | 24 2 | 2 3 | 31 0 | 24 2 | 0 8 |
| L 11 | 1 66 | 11 0 | 19 9 | 13 0 | 1 4 | 44 2 | 19 5 | 2 0 |
| L 12 | 1 84 | 12.2 | 25 1 | 15 9 | 2 9 | 27 0 | 25 5 | 3 6 |
| L 13 | 1 27 | 10 6 | 25 4 | 13 1 | 3 8 | 30 1 | 25 5 | 2 1 |
| L 14 | 1 10 | 8 4 | 26 3 | 14 0 | 1 3 | 31 2 | 25 6 | 1 6 |
| L 16 | 1 63 | 9 5 | 27 0 | 14 0 | 1 4 | 34 7 | 22.1 | 0 4 |
| Mean | 1 44 | 10 3 | 23 1 | 15 5 | 1 7 | 33 8 | 24 1 | 1 5 |
| ±S D | 0 2 | 1 1 | 2 9 | 4 3 | 0 9 | 5 4 | 2 0 | 0 8 |
| Normal | 1 11 | 10 0 | 29 0 | 14 0 | 1 6 | 27 4 | 25 9 | 2 1 |
| ±S D | 0 11 | 0 7 | 2.2 | 1 7 | 0 4 | 1 5 | 1 7 | 1 1 |

b talipoproteinemia with serum cholesterol valu s b tween 391 and 700 mg/dl (Table VII) all had normal erythrocyte cholesterol total phospholipids and phospholipid pattern (Table VIII)

To test the possibility that inhibitors of LCAT were present in plasma of patients with obstructive jaundice the exp riment shown in Table IX was p rformed When plasma from a normal individual and the patient L 17 were mixed in the ratios 2 1 and 1 1 no inhibition of the LCAT activity was obtained

## DISCUSSION

Free cholesterol and to a lesser degree lecithin of plasma lipoproteins exchange with erythrocyte cholesterol and lecithin (2 3 5 6 10) Patients with a-α lipoproteinemia (Tangier disease) and patients with a β lipoproteinemia (acanthocytosis) have reduced levels of total cholesterol in plasma. The ratio of free/esterifi d cholesterol however is normal The erythrocyte cholesterol in these two diseases is normal (17 18 21)

In patients with hyper β lipoproteinemia the red cell cholesterol is normal (Tables VII and VIII) Red cell cholesterol is normal or low also in other hyp rlip mic states (12) Thes data may mean that the erythrocyte cholesterol is relatively unaffected by the concentration of plasma lipoproteins if the lipid composition of the lipoproteins is normal with respect to the ratio free/esterified cholesterol

In both a-α and a β lipoproteinemia there are abnormal patterns of phospholipids in plasma and some of the abnormalities in the phospholipid composition of the erythrocytes are reflections of these changes (7 18 21)

In familial LCAT deficiency both the ratio of esterified to unesterified cholesterol and the phospholipid pattern in plasma are abnormal (8 13) Thes patients have highly abnormal lipid com

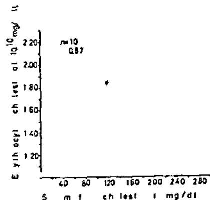


*Fig 1* Correlation between erythrocyte cholesterol and serum free cholesterol in patients with chronic hepatitis and liver cirrhosis.

Table IVa *Patients with obstructive jaundice Clinical and hematological data*

| Patients No | Sex | Age (y) | Diagnosis | Duration of symptoms | Hb (g/100 ml) | RBC (mill/μl) | WBC (/μl) | Platelets (/μl) | ESR (mm/h) | Reticulocytes (/1000 red cell) | Serum iron (μg/100 ml) | Transferrin (μg/100 ml) | Osmotic fragility |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| L 1 | ♂ | 62 | Pancreatitis chron | 4 y | 77 (11 4) | 3 96 | 4 900 | 278 000 | 79 | | 115 | 400 | Decreased |
| L 2 | ♀ | 74 | Cholangiohepatitis Occlusio duct choledochi | 6 mo | 71 (10 5) | 3 67 | 11 000 | 281 000 | 124 | 44 | 63 | 248 | Decreased |
| L 8 | ♂ | 47 | Pancreatitis chron Cholangitis Cirrhosis biliaris | 3 y | 90 (13 3) | 4 40 | 7 500 | 278 000 | 127 | 13 | 40 | | Decreased |
| L 9 | ♂ | 65 | Cancer pancreatis | 1 mo | 71 (10 5) | 3 65 | 10 400 | 240 000 | 55 | 2 | 138 | 340 | Slightly decreased |
| L 10 | ♂ | 72 | Cholangitis sclerotic | 6 mo | 69 (10 2) | 3 30 | 11 500 | 366 000 | 15 | 27 | 40 | | Decreased |
| L 17 | ♂ | 50 | Cholelithiasis | 1 mo | 80 (11 9) | | 9 900 | | 41 | | 135 | 170 | Decreased |

Table V *Patients with obstructive jaundice Plasma lipid values lipoprotein pattern and LCAT activity*

Abbreviations as in Tabl II

| Pat no | Plasma lipids: Cholesterol Total (mg/dl) | Cholesterol Free (mg/dl) | Cholesterol Esterif ( ) | Triglycerides (mg/dl) | Phospholipids Total (mg/dl) | Per cent of total P E | P S | L | S | L L | Lipoprotein electrophoresis | LCAT activity ( F C est /h) | LCAT activity (mμmole/ml/h) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| L 1 | 240 | 185 | 23 | 196 | 286 | 3 5 | 0 7 | 86 4 | 8 3 | 1 1 | Broad β band No α band | 0 11 | 5 |
| L 2 | 580 | 478 | 18 | 246 | 413 | 2 3 | 0 2 | 86 3 | 9 8 | 1 4 | Very broad β band No α band | 0 07 | 9 |
| L 8 | 423 | 323 | 24 | 214 | 511 | 3 2 | 0 3 | 88 3 | 7 2 | 1 0 | Broad β band No α band | 0 14 | 12 |
| L 9 | 266 | 120 | 55 | 128 | 212 | 1 8 | 1 1 | 84 7 | 11 1 | 1 3 | Normal | 0 43 | 14 |
| L 10 | 504 | 406 | 19 | 147 | 651 | 2 4 | 0 4 | 88 9 | 6 7 | 1 6 | Broad β band with trailing No α band | 0 01 | 1 |
| L 17 | 131 | 106 | 19 | 250 | 168 | 3 2 | 1 4 | 62.7 | .6 9 | 5 8 | β lp normal No α band | 0 07 | 2 |
| Mean | 357 | 270 | 26 | 197 | 373 | 2 7 | 0 7 | 82 9 | 11 7 | 2 0 | | 0 14 | 7 2 |

Table IVb *Patients with obstructive jaundice Liver function tests*

| Pat no | SGOT (U/l) | SGPT (U/l) | Alk. phosph (U/l) | Bilirubin (mg/dl) | Thymol turbidity (U) | Normotest (% of normal) | Thrombotest (% of normal) | Serum Albumin (g/dl) | Serum globulin (g/dl) |
|---|---|---|---|---|---|---|---|---|---|
| L 1 | 87 | 44 | 360 | 14 0 | 0 03 | 100 | 86 | 2 5 | 1 7 |
| L 2 | 72 | 39 | 105 | 10 8 | 0 03 | 48 | 15 | 2.3 | 1 7 |
| L 8 | 56 | 35 | 558 | 12 0 | 0 23 | 27 | 56 | 2 5 | 3 3 |
| L 9 | 81 | 110 | 226 | 8 2 | 0 00 | 82 | 58 | 3 0 | 1 1 |
| L 10 | 111 | 50 | 171 | 18 0 | 0 06 | 100 | 72 | 2 7 | 3 5 |
| L 17 | 52 | 35 | 105 | 25 0 | 0 05 | | 15 | 2 6 | 2 3 |
| Mean | 77 | 52 | 254 | 14 6 | 0 07 | | 50 | 2.6 | 2.3 |

position of their erythrocytes high cholesterol and lecithin and low sphingomyelin and phosphatidyl ethanolamine The abnormal plasma and red cell lipid pattern in these patients most probably reflect the complete lack of LCAT activity in plasma (7 14)

In the present investigation we have compared plasma and erythrocyte lipids in patients with liver disease and have found that increased erythrocyte cholesterol content is present in patients with chronic parenchymatous liver disease A significant decrease in esterification of plasma cholesterol was found and red cell cholesterol content and plasma free cholesterol level were highly significantly correlated in these patients It is reasonable to believe that this is a consequence of the demonstrated low levels of LCAT activity

The erythrocyte lipid composition of patients with obstructive jaundice deviated markedly from normal The cholesterol content was nearly twice the normal value The total phospholipids of the red cells were slightly increased mainly due to an increase in lecithin while sphingomyelin and phospatidyl ethanolamine had lower concentration than normal

The studies of erythrocyte lipids in liver disease (1 11 22) agree that erythrocyte cholesterol is elevated Also the total phospholipid may be elevated to some degree with abnormal phospholipid pattern Our studies confirm these findings The mechanism for the altered erythrocyte lipid composition is however not clear

Cooper and Jandl (2) suggest that in obstructive jaundice the changes in red cell lipids may partly be due to bile salt influence in the plasma/cell partition of free cholesterol This suggestion cannot explain the findings in our patients with chronic parenchymatous liver disease as they had no marked biliary obstruction

The increased red cell cholesterol may be a reflection of the abnormal ratio of esterified to unesterified cholesterol due to the decreased ac

Table VI *Patients with obstructive jaundice Erythrocyte lipids*
Abbreviations as in Table II

| Pat. no | Cholesterol ($10^{-1}$ mg/cell) | Phospholipids Total ($10^{-13}$ mg/cell) | Per cent of total P E | P S | P I | L | S | L L |
|---|---|---|---|---|---|---|---|---|
| L 1 | 2.43 | 11 4 | 26 3 | 8 4 | 0 6 | 44 8 | 19 2 | 0 7 |
| L 2 | 2 27 | 13 6 | 18 9 | 11 6 | 0 8 | 50 1 | 18 2 | 0 4 |
| L 8 | 1 67 | 12.4 | 17 3 | 18 2 | 2.6 | 44 2 | 16 3 | 1 4 |
| L 9 | 1 47 | 10 0 | 21 5 | 14 6 | 2 1 | 36 1 | 24 6 | 1 1 |
| L 10 | 1 46 | 12.2 | 20 3 | 14 8 | 1 1 | 46 7 | 15 8 | 1 3 |
| L 17 | 1 84 | 12 8 | 18 7 | 16 8 | 1 9 | 40 0 | 19 4 | 3 2 |
| Mean | 1 86 | 12 1 | 0 5 | 14 1 | 1 5 | 43 7 | 18 9 | 1 3 |
| ±S D | 0 40 | 1 3 | 3 1 | 3 5 | 0 9 | 5 0 | 3 0 | 0 9 |

Table VII *Patients with primary hyperbetalipoproteinemia Plasma lipid values*
Abbreviations as in Tabl II

| | Cholesterol | | | | Phospholipids | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Per cent of total | | | | |
| Pat no | Total (mg/dl) | Free (mg/dl) | Esterif ( ) | Triglycerides (mg/dl) | Total (mg dl) | P E | P S | L | S | L L |
| F 4 | 583 | 161 | 72 | 140 | 300 | 2 9 | 2 6 | 70 2 | 21 5 | 2 8 |
| F 11 | 675 | 194 | 72 | 135 | 269 | 4 9 | 2 7 | 65 7 | 23 7 | 3 0 |
| F 16 | 700 | 188 | 73 | 110 | 296 | 4 1 | 2 5 | 69 2 | 22 0 | 2 2 |
| F 24 | 391 | 110 | 72 | 70 | 151 | 3 8 | 2.6 | 67 1 | 22 8 | 3 3 |
| F 25 | 630 | 176 | 72 | 84 | 246 | 5 5 | 2 5 | 72 3 | 16 7 | 3 0 |

Table VIII *Patients with primary hyperbetalipoproteinemia Erythrocyte lipids*
Abbreviations as in Table II

| Patients | | | | | Erythrocyte lipids | Phospholipids | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Per cent of total | | | | | |
| No | Sex | Age | Hb ( ) | RBC (mill/µl) | Cholesterol ($10^{-10}$ mg/cell) | Total ($10^{-10}$ mg/cell) | P E | P S | P I | L | S | L L |
| F 4 | ♂ | 59 | 97 | 5 30 | 1 17 | 9 7 | 27 6 | 14 7 | 0 5 | 30 6 | 24 8 | 1 8 |
| F 11 | ♀ | 16 | 84 | 4 22 | 1 30 | 9 5 | 28 2 | 18 3 | 0 8 | 25 9 | 25 6 | 1.2 |
| F 19 | ♂ | 30 | 90 | 4 73 | 1 13 | 10 8 | 24 3 | 11 3 | 4 3 | 38 1 | 21 1 | 0 9 |
| F 24 | ♀ | 23 | 75 | 3 90 | 0 93 | 8 8 | 23 3 | 15 5 | 3 0 | 29 2 | 26 9 | 2.1 |
| F 25 | ♂ | 51 | 86 | 4 10 | 0 95 | 11 5 | 24 6 | 12 8 | 4 5 | 27 7 | 29 9 | 2 5 |

Table IX *Influence of icteric serum on LCAT activity in normal serum*

| Serum in incubation mixture (µl) Normal | Icteric | Labelled cholesterol esterified per hour ( ) | Cholesteryl esters formed per ml serum mixture per hour (mµ moles) | Cholesteryl esters formed per ml of normal serum per hour (mµ moles) |
|---|---|---|---|---|
| 100 | 0 | 2 85 | 48 | 48 |
| 0 | 100 | 0 | 0 | — |
| 75 | 25 | 2 02 | 39 | 52 |
| 50 | 50 | 1 13 | 26 | 53 |

*The concentration of unesterified cholesterol in normal and icteric serum was 1 66 mM and 2 64 mM respectively Total incubation mixture contained in the preincubation period 100 µl serum 30 µl of $^{14}$C-cholesterol bound to 5 albumin in 0 2 M phosphate buffer pH 7 3 (corresponding to about 120 000 cpm) 40 µl of 0 01 M 5 5 -dithiobis (2 nitrobenzoic acid) in 0 2 M phosphate buffer pH 7 3 The esterification reaction was started after 4 hours with 20 µl of 0 1 M mercaptoethanol. Incubation for one hour at 37°*

tivity of LCAT in plasma in this group of patients In liver failure the reduced plasma esterification activity has been explained by reduced liver biosynth sis of the LCAT as this enzyme most probably is synthesized by the liver (9) Our data from patients with chronic hepatitis/cirrhosis may confirm this assumption

In biliary obstruction the increased red cell cholesterol may not be due solely to the demonstrated low plasma LCAT activity As suggested by Cooper and Jandl (2) an increase in plasma bile salts may increase the shift of free cholesterol from plasma to cells in these patients Furth r more the reason for the low LCAT activity is not clear It has been proposed that the plasma bile salts inhibit the plasma LCAT (2) Our results (Table IX) are not fully consistent with this assumption When normal plasma and severe icteric

plasma with almost no LCAT activity are mixed in equal amounts, the LCAT from normal plasma is not inhibited The almost complete lack of LCAT activity in patients with severe obstructive jaundic is therefore most probably not due to plasma inhibitors of the LCAT p-r se It may be due to inhibited production of LCAT in the liver inhibited release of the enzyme from the liver or inhibited activation of the enzyme in plasma Furth r investigations are needed to see which of these explanations if any is the most possible

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## REFERENCES


1 Boon J Broekhuyse R M van Munster P & Schretlen, E. Clin. chim. Acta 23 453 1969
2. Cooper R. A. & Jandl, J H J clin. Invest. 47 809 1968
3 — J clin. Invest. 48 906, 1969
4 Courchaine A J., Miller W H & Stein, D B Jr Clin. Chem. 5 609 1959
5 Firkin B G & Wiley J S Progr Hemat, 5 26 1967
6. de Gier J Red cell lipids. In Walsh, R. J & Pitney W R. Plenary Sessions XIth Congress of the International Society of Haematology C N Blight Sydney 1966
7 Gjone E., Torsvik, H & Norum, K. R. Scand J clin. Lab Invest. 21 327 1968
8 Gjone E. & Norum, K. R. Acta med scand 183 107 1968
9 Glomset J A. J Lipid Res 9 155 1958
10 Murphy J R. J Lab clin. Med. 65 7 6 1965
11 Neerhout, R. C J Lab clin. Med. 71 438 1968
12 — J Lab clin. Med. 71 448 1968
13 Norum, K. R. & Gjone E. Scand, J clin. Lab Invest 20 231 1967
14 — Scand. J clin. Lab Invest. 22 94 1968
15 Norum, K. R. & Stokke K. T In preparation.
16 Parker F & Paterson, N F J Lipid Res. 6 455 1965
17 Phillips, G B J Lab clin. Med. 59 357 1962.
18 Shacklady M M., Djardjouras, E. M & Lloyd, J K. Lancet 2 151 1968
19 Skipski, V P., Petersen N F & Barclay Marion Biochem. J 90 374 1964
20 Ways, P & Hanahan, D J J Lipid Res 5 318 1964
21 Ways, P Reed C F & Hanahan, D J J clin Invest. 4 1248 1963
22. Westerman, M P., Balcerzak, S P & Heinle E W J Lab clin. Med. 72 663 1968

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# STUDIES IN SUBJECTS WITH POSITIVE POSTPRANDIAL CLINISTIX® TEST

## I Serum Insulin like Activity (SILA) and Free Cortisol in Newly Discovered Diabetics

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*Abstract* In a diabetes detection survey based on post prandial Clinistix® testing, 2477 out of 228 833 participants (1 08%) reacted positively In 2180 a 3 hour oral glucose tolerance test was performed Diagnostic criteria for diabetes were established in a random sample from the same population matched in age and sex The controls were healthy and had no known heredity for diabetes The requirement for a diagnosis of diabetes was that all 10 capillary blood sugar values during the tolerance test should exceed the corresponding mean plus 3 S D for the control group Likewise a normal tolerance was only considered present when none of the 10 values reached the mean plus 2 S D for the control group

A diagnosis of diabetes was made in 538 subjects i e 0 2 of the population under study

Fasting SILA values in 16 normal men less than 50 years of age showed a mean of 94 μU per ml and in 12 men more than 50 a mean value of 83 μU per ml was found Fasting SILA values in eight normal women less than 50 showed a mean of 34 μU per ml and in nine women more than 50 a mean of 75 μU per ml was found. The low value in women less than 50 was significantly different ($p < 0 001$) from the male group and also from values found in women more than 50 years of age ($p < 0 01$)

Fasting SILA in 16 men and 14 women with newly discovered diabetes and age more than 50 showed all values in the normal range Three diabetic women less than 50 had 50 61 and 146 μU per ml

SILA during the glucose tolerance test in six patients with diabetes showed a subnormal response in all of them

SILA during the glucose tolerance test in five subjects considered diabetics by conventional criteria but not according to our criteria, showed a normal response

Fasting serum corticosteroids in six diabetic subjects were significantly higher than in five controls

Serum corticosteroids during the glucose tolerance test dropped to the same base level both in the diabetic and in the normal group

A diabetes detection campaign covering 82 of the population was conducted in Malmohus County in Southern Sweden from 1962 to 1965 The screening procedure was Clinistix® testing of the urine collected after a carbohydrate rich meal (1) As a basis for the diagnosis of diabetes the criteria suggested by Klimt et al (7) and by Fajans and Conn (3) were used When the study was under way it became clear that proper evaluation of the results required control samples from the same population matched with respect to age and sex In an attempt to obtain the most accurate information possible from the oral glucose tolerance test the mean plus three standard deviations for the control group was selected as the boundary between the diabetic group and a borderline group The mean plus two standard deviations represented the boundary between the normal group and the borderline group

Further studies were necessary to justify these criteria The present report deals with subjects diagnosed as previously unknown diabetics

## MATERIAL AND METHODS

The survey covered 228 833 participants They collected the urine passed two hours after a carbohydrate rich meal Those showing a positive Clinistix® test were admitted for a 3 hour oral glucose tolerance test An oral glucose dose of 30 g/m body surface was given as a 10 solution at 9 a.m with the subject fasting since the previous evening.

Capillary blood glucose determinations according to Marks (12) were performed fasting and after 15 30 45 60 75 90 120 150 and 180 min The patient rested in

Table I *Control material*

Blood glucose values (mg/100 ml) ± S.D. during oral glucose tolerance test (30 g glucose in 10 % solution per 1 $m^2$ body surface) in 142 females and 121 males without diabetic heredity

| | Females, age group (y.) | | | Males, age group (y.) | | |
|---|---|---|---|---|---|---|
| Min | ≤39 | 40–59 | ≥60 | ≤39 | 40–59 | ≥60 |
| 0 | 75.8± 6.0 | 78.9± 8.1 | 82.0± 9.8 | 77.4± 5.5 | 80 ± 9.5 | 81.0± 9.1 |
| 15 | 129.5±23.5 | 138.8±16.4 | 145.7±22.3 | 131.9±16.0 | 146 ±18.7 | 141.4±23.9 |
| 30 | 147.6±25.9 | 166.7±20 | 184.3±30.4 | 160 ±21.9 | 174.5±27.2 | 176 ±30.7 |
| 45 | 145.4±30.1 | 163.3±31.5 | 195.2±31.6 | 145.9±28.6 | 173.5±32.6 | 179.3±30.7 |
| 60 | 133.7±32.7 | 151.1±34.3 | 188.8±37.1 | 127.4±28.2 | 158.8±35.6 | 170.3±38.4 |
| 75 | 118 ±22 | 124.1±27.3 | 162.7±40 | 109.3±25.4 | 129.3±40.4 | 148.4±35.6 |
| 90 | 116.5±20 | 122.6±32.6 | 150.5±42.0 | 100.5±22.5 | 110.8±37.9 | 134.5±34.8 |
| 120 | 99.6±16.4 | 99 ±23.6 | 123.2±40.2 | 87 ±21.6 | 86.1±27.6 | 103.6±29 |
| 150 | 81.4±17.5 | 76.7±13.9 | 87.6±29 | 71.3± 9.3 | 71.1±15.7 | 77 ±19.5 |
| 180 | 72.5±10.6 | 70.3± 8.5 | 73.4±15.1 | 70.3± 9.4 | 68.3±11.1 | 68.2±10.3 |
| No. of subjects | 39 | 71 | 32 | 49 | 41 | 31 |

a chair during the test. Urine was tested with Clinistix® fasting and after one, two and three hours. 538 subjects fullfilled the criteria for diabetes. The newly discovered diabetics represented 0.2% of the population.

From this group 16 men with a median age of 57 years (24–74) and 17 women with a median age of 61 years (35–76) were selected at random for the present studies.

The normal controls were selected from the county population register amongst those over 16 years of age—90 names were originally drawn, representing all persons born on January 20 and July 20 and from this total, 315 subjects were taken at random. Diabetes was known in the families of 52 (16%); these were excluded. The remaining controls went through a physical and laboratory examination. The results of the oral glucose tolerance tests are given in Table I for 263 subjects who up to the present study had been examined.

Serum insulin-like activity (SILA) was determined in 28 men and 17 women with normal glucose tolerance and no glucosuria during the tolerance test. In addition, six men and seven women also with a normal tolerance but with diabetes in their families were studied. A positive family history was considered documented only when at least one case of diabetes was known in a close relative such as parents, siblings, children, grandparents, aunts, or uncles.

*Determination of serum insulin-like activity (SILA) in undiluted serum*

The rat epididymal fat pad technique (13, 15, 16) as modified by Lyngsøe (10, 11) was employed. The assays were carried out as multiple three point designs, in which four serum samples were run concurrently against two doses of standard insulin. For statistical analysis the values of SILA were converted to logarithms. The result was expressed in arithmetical values as mean and mean range (i.e. the mean ± standard error of the mean calculated on the logarithmic basis). Blood was collected by venous puncture in the fasting state or during oral glucose tolerance tests after 30, 90 and 150 min. The blood was allowed to clot at room temperature and then centrifuged. Serum was removed and kept frozen at −20 C until examined. It was thawed at room temperature and immediately assayed. Control determinations on serum kept frozen at various intervals were made with 27 paired samples kept for up to two months. No significant change of activity was found. The mean index of precision (17) for all assays during the investigation was 0.4.

*Determination of serum free corticosteroids*

The serum corticosteroid determinations were performed by the fluorometric method of Guillemin et al. (5).

*Amount of body fat*

Body fat was estimated according to von Döbeln ( ). In subjects in which anthropometric measurements had not been made the weight in kilograms/(height in metres)$^2$ quotient (14) was used. A comparison between this quotient and the expression obtained by von Döbeln's method was made in 165 subjects. A highly significant ($p < 0.001$) correlation ($r = 0.86$) was found.

## RESULTS

### A. SILA in the Control Group

*(a) Fasting*

Fig. 1 presents the results. There were 16 men and eight women less than 50 years of age. SILA in the male group gave a mean value of 94 μU per ml with a mean range of 86–103. The eight females showed a mean of 34 μU per ml with a mean range of 27–43. The difference between the men and women was highly significant ($p < 0.001$).

In 21 subjects above 50 years of age 12 men showed a mean SILA of 83 μU per ml with a mean range of 73–94, and nine women a mean

of 75 μU per ml with a mean range of 67–83 In this age group there was no sex difference Fig 1 also includes fasting SILA values in 13 subjects with a normal glucose tolerance and heredity for diabetes Ten subjects were less than 50 years of age Six men had a mean value of 108 μU per ml with a mean range of 94/124 slightly higher but not significantly increased above the control male group of the same age Four females had a mean SILA value of 50 μU per ml with a mean range of 41–60 This was also slightly higher than in the corresponding control group Also in this group with normal glucose tolerance and heredity for diabetes a significant difference ($p < 0.001$) was found between men and women Only three female subjects above 50 years of age were studied They had a remarkably high SILA value with a mean of 128 μU per ml SILA values showed no correlation to fasting blood glucose No correlation was found between SILA and age or body weight but there was a slight correlation between SILA and per cent body fat for males $r = 0.6867$ ($p < 0.01$) for females $r = 0.6254$ ($p < 0.05$)

The normal females less than 50 years of age who showed a significantly lower SILA value than the males of the same age and than the females above 50 had a calculated body fat of 24.6% The females above 50 had a calculated body fat of 30.1% The difference between females less than and above 50 years of age was significant ($p = 0.025$)—low body fat and low SILA in females less than 50 years of age

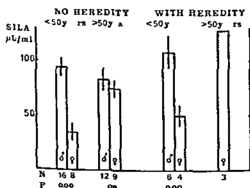



*Fig 1* Fasting SILA (mean and mean range) in 45 control subjects without diabetic heredity and in 13 control subjects with known diabetic heredity *P* = probability level of the random difference

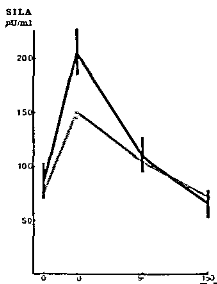



*Fig 2* SILA (mean and mean range) during oral glucose tolerance test in six control subjects without diabetic heredity (———) and in four control subjects with known diabetic heredity (|||||)

## (*b*) *SILA during the oral glucose tolerance test*

Fig 2 shows the rise in SILA in four males and two females in the control group These subjects were all more than 50 years The mean and mean range is given

The figure also gives the SILA response in four subjects with a normal glucose tolerance but with known heredity for diabetes At 30 min this curve was lower than in the control group but at 90 and 150 min there was no difference

No sex difference was found in these studies but it included no females less than 50 years of age these will be the object of a separate study

## B *SILA in Subjects with Newly Discovered Diabetes*

### (*a*) *Fasting*

In Fig 3 the fasting SILA values for 16 newly discovered male diabetics are compared with the fasting SILA values from the 28 subjects in the control group The mean for the diabetic group was 113 μU per ml with a mean range of 100–127 The increase was not significant ($p > 0.05$)

In the female group of newly discovered diabetics three were less than 50 years of age Their fasting SILA values were 50 61 and 146 μU per ml which should be compared with the mean of 34 μU per ml and the mean range of 27–43 for

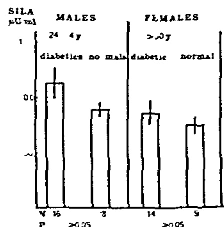



*Fig 3* SILA (mean and mean range) in 16 diabetic males and in 14 diabetic females as compared with that of 28 male control subjects and nine female control subjects. *P* = probability level of the random difference

the corresponding control group There were 14 females above 50 years of age in the group of newly discovered diabetics Their fasting SILA values (Fig. 3) showed a mean of 86 with a mean range of 76–98 μU per ml, which was not significantly increased above the values for the control group ($p > 0.05$)

No correlation was found in subjects with newly discovered diabetes between the SILA value and fasting blood glucose age body weight, or per cent body fat.

*(b) SILA during the oral glucose tolerance test*

Fig. 4 shows the SILA response in six newly discovered diabetics two males aged 41 and 62

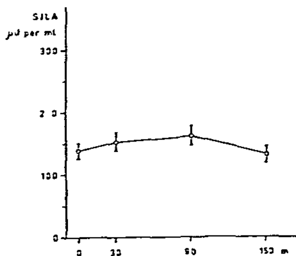



*Fig 4* SILA (mean and mean range) during oral glucose tolerance test in six subjects with newly discovered diabetes.

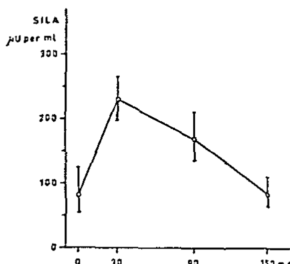



*Fig 5* SILA (mean and mean range) during oral glucose tolerance test in five subjects diabetic according to Brandt et al. (1) but non-diabetic according to present criteria.

and four females aged 56 to 69 years There was no significant rise

Fig. 5 shows the SILA values in five subjects who according to our previous criteria (1) should have been labelled diabetics but according to our present criteria are non-diabetic The response agrees with the response seen in the normal controls (Fig. 2) and not with the unresponsiveness found in the diabetic group (Fig. 4)

### C *Serum Free Corticosteroids*

Serum corticosteroid determinations were made fasting and during the oral glucose tolerance test in five normal subjects and six subjects with newly discovered diabetes. The same serum samples were used for the determination of SILA and serum free corticosteroids Fasting corticosteroids were significantly higher in the diabetic group than in the normal controls ($p < 0.05$) During the glucose tolerance test there was a rapid and significant decrease in corticosteroid level It was more marked in the diabetic group than in the normal controls (Fig. 6)

## DISCUSSION

In the present study criteria for the diagnosis of diabetes were established in a normal group selected at random from the population The newly discovered diabetics, traced by a postprandial

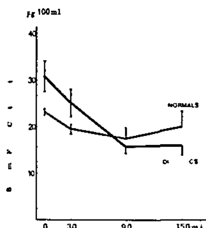



*Fig 6* Serum free corticosteroids (mean ± standard error of the mean) during oral glucose tolerance test in five control subjects (||||||) and in six subjects with newly discovered maturity onset diabetes (———)

positive Clinistix® test, amounted to 0 2 % of the population

When compared with reported findings in diabetic campaigns this low figure prompted further studies The strict criteria used in the evaluation of the oral glucose tolerance test have not been applied previously as far as we are aware The justification for such treatment could be illustrated by comparing the insulin response to glucose in a group that, by previous criteria should be regarded as diabetic with the diabetic and normal groups in the present study SILA values in such a group normal by our present criteria but diabetic according to our previous criteria (1) showed a response typical of normal non-diabetic subjects Although the present series are small they support the criteria used

The most remarkable observation in the normal controls was the low SILA values in women less than 50 years of age Women more than 50 and men of all ages were not significantly different from one another Lyngsøe (11) found no variation with regard to sex or age in SILA determined in undiluted serum In diluted serum however there was a tendency to low values in women particularly in those less than 40 years of age

In our studies SILA showed no correlation to fasting blood glucose or to body weight There was a slight correlation to body fat $r = 0\ 6867$ for males and $r = 0\ 6254$ for females The females less than 50 had a lower body fat percentage 24 6 % than the women more than 50 who had a fat percentage of 30 1 % and this difference was significant ($p = 0\ 025$)

In normal subjects with a diabetic heredity fasting SILA values were higher than in the control group but the difference was not statistically signifcant SILA during the glucose tolerance test in this group however gave a significantly lower response than in the normal controls which suggests a diabetic pattern

The study of serum corticosteroids was initiated by the observation (9) that physiological amounts of cortisol are able to suppress insulin induced glucose metabolism in the rat epididymal adipose tissue in vitro A high cortisol level in vivo might also interfere with the fat pad assay for insulin like activity in serum The high fasting levels of immunoreactive insulin during the early stage of maturity onset diab tes (6) contrast with our observations of normal SILA values This discrepancy could be due to a high cortisol level Whether the high cortisol values observed in the fasting state actually interfere with glucose utilization in the adipose tissues of man remains to be further studied Elevated cortisol levels in blood and urine of diabetic subjects have been reported (4 8) Finally the technique for evaluation of the oral glucose tolerance test that we have adopted is supported by the observations of a subnormal SILA response in subjects with recently discovered diabetes a pattern of a similar type in normal subjects with known diabetes in the family and a normal SILA response in subjects who according to our criteria for the glucose tolerance test should be labelled normal although by previous criteria they should be regarded as diabetics

## REFERENCES


1 Brandt, L., Norden A., Schersten, B & Trydin., N A diabetes detection campaign in southern Sweden Results of 69 000 examinations. Acta med scand 1 6 555 1964

2 von Dobeln, N Anthropometric determination of fat free body weight Acta med scand. 165 37 1959

3 Fajans, S S & Conn J W An approach to the prediction of diabetes mellitus by modification of the glucose tolerance test with cortisone Diabetes 3 296, 1954

4 Goth A., Leugyel, L Savely C., Bartha, M & Lorincz, L The role of pituitary adrenal system in the physiopathology of diabetes III Z Vitamin Hormon u. Fermentforsch. 10 15 1959

5 Guillemin, R., Clayton, G W & Lipscomb H. S. Fluorometric measurement of rat plasma and adrenal corticosterone concentration. J Lab clin. Med. 53 830 1959
6 Hales, C. N & Randle P J Effects of low-carbohydrate diet and diabetes mellitus on plasma concentrations of glucose non-esterified fatty acid and insulin during oral glucose-tolerance tests. Lancet 1 790 1963
7 Klimt, C R., Wolff F W., Silverman, C & Conant, J Calibration of a simplified cortison glucose tolerance test. Diabetes 10 351 1961
8 Lentle, B C & Thomas, J P Adrenal function and the complications of diabetes mellitus. Lancet 1 544 1964
9 Lundquist, I. On the significance of serum dilution and cortisol antagonism in the rat fat pad bioassay of insulin. Acta endocr Kbh. 58 11 1968
10 Lyngsøe, J Determination of the insulin like activity in serum using rat epididymal adipose tissue Scand. J clin. Lab Invest. 13 6-8 1961
11 — The insulin-like activity in serum determined by the rat epididymal fat method. I. Normal values in undiluted and diluted serum, and the effect of ingestion of glucose. Acta med. scand. 171 365 1962
12 Marks, V An improved glucose oxidase method for determining blood, CSF and urine glucose levels. Clin. chim. Acta 4 395 1959
13 Martin, D B., Renold, A. E. & Dagenais, Y M. An assay for insulin like activity using rat adipose tissue. Lancet 2 76 1958
14 Nicholson, J P & Zilva, J F Body constituents and functions in relation to height and weight. Clin. Sci. 27 97 1964
15 Renold, A. E., Martin, D B., Dagenais, Y M., Steinke, J., Nickerson, R. J & Sheps, M C Measurement of small quantities of insulin like activity using rat adipose tissue I. A proposed procedure J clin. Invest. 39 1487 1960
16 Sheps, M C., Nickerson, R. J., Dagenais, Y M., Steinke, J., Martin, D B & Renold, A. E. Measurement of small quantities of insulin-like activity using rat adipose tissue II Evaluation of performance. J clin. Invest. 39 1499 1960
17 Snedecor G W Statistical methods, fifth ed. The Iowa State University Press, Ames, Iowa 1956

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# STUDIES IN SUBJECTS WITH POSITIVE POSTPRANDIAL CLINISTIX® TEST

## II Serum Insulin-like Activity (SILA) in Non-diabetic Glucosurics

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*Abstract* SILA has been determined in two groups of subjects with a non-diabetic positive Clinistix® test, one with a normal glucose tolerance ("renal glucosuria") and the other with a high initial blood glucose rise and normal level after 60 min (alimentary glucosuria or "oxyhyperglucemia")

Nineteen males with *constant renal glucosuria* showed normal fasting SILA. Seven males in this group were studied during oral glucose loading, four subjects showed a low ("diabetic") SILA response while three showed a normal response Three females with constant glucosuria had higher than normal fasting SILA two of them were studied during oral glucose loading and in both the SILA response was normal.

Ninety males with *intermittent renal glucosuria* had normal fasting SILA, but sixteen females had higher than normal fasting SILA. Of the ninety twenty were studied during glucose loading, fifteen had a normal SILA response whereas five had subnormal ("diabetic type") response

Fourteen males with *oxyhyperglucemia* had fasting SILA above normal, and three out of four females had increased fasting SILA. The SILA response to glucose was normal in the five males studied, but the insulinogenic index was lower than normal.

It is concluded that the high fasting SILA and subnormal "diabetic type" SILA response to glucose loading frequently found in non-diabetic glucosurics may indicate that non-diabetic glucosuria in some cases is related to an increased risk of developing diabetes.

"Non-diabetic glucosuria" represents a poorly defined condition frequently encountered in diabetic surveys when testing of the urine for glucose is used as screening method Two mechanisms are presumed to be involved one is related to renal factors and the "renal threshold" for glucose and the other to the rapid emptying of the stomach leading to a rise in blood glucose above the renal threshold The first condition is known as "renal glucosuria" and the second as "alimentary glucosuria" or "oxyhyperglucemia" the latter term referring to the rapid ("oxy" greek for sharp) rise of the blood sugar

Marble (10) required for the diagnosis of renal glucosuria that all urinary specimens fasting or after meals should contain sugar Lawrence (7) accepted cases of renal glucosuria whenever a positive sugar reaction was observed in the urine in the presence of a normal glucose tolerance even if samples obtained fasting were negative—so-called intermittent glucosuria in contrast to the constant glucosuria required for the diagnosis by Marble Whether such distinctions represent any true differentiation depends on among other things the technique for measuring glucose in the urine Up to now methods for measuring urinary glucose have been crude and unprecise The routine procedure in screening for diabetes by test papers such as Clinistix® however has been commonly used This was also the technique employed in the survey which formed the basis for the present studies We therefore felt obliged to investigate subjects with non-diabetic glucosuria further first of all to collect, if possible information about any relation to diabetes.

In a previous report serum insulin like activity (SILA) studied by the epididymal fat pad technique was described in newly discovered diabetics and in normal subjects (9) For the diagnosis of diabetes rigorous criteria were used—all of ten blood glucose values should be above the mean plus 3 S.D. for the age and sex matched control group A characteristic lack of SILA response to glucose in subjects with diabetes was observed

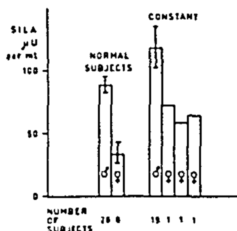



*Fig 1* Fasting SILA (mean and mean range) in constant renal glucosuria.

In an attempt to find some relation between diabetes and non-diabetic glucosuria techniques previously described were applied in the present studies.

## MATERIAL AND METHODS

The clinical material consisted of 1 8 subjects selected at random from the group of non-diabetic glucosurics [illegible] during the diabetes survey which has previously been described (1, 9)

During the tolerance test the urine was tested with Clinistix® before giving the glucose and again after one, two and three hours. "C[illegible]stant glucosuri[illegible]" had a positive outcome of the Clinistix® test in all four samples. "*intermittent glucosurics*" were positive in one or more samples, but not in all four

The material was further divided depending upon the blood glucose response into one group with all ten values below the mean plus 2 S.D. "*renal glucosuria*" and into a second group in which the values after 60 min were below the mean plus 2 S.D., but the values during the first 60 min were above the mean plus 2 S.D., "*oxyhyperglucemia*"

Methods for the glucose tolerance test, blood glucose determinations and determinations of SILA have already been described (9)

## RESULTS

### *Renal Glucosuria*

#### *Constant glucosuria*

Fasting SILA determinations in 19 males with a median age of 46 years (19–77 years) and three females (19, 45 and 48 years old) are illustrated in Fig 1. The mean for the male group was 119 μU per ml with a mean range of 103 to 136 μU per ml. Although increased above the values for the corresponding control group the difference was not statistically significant ($p >$ 0.05). The three females had 73, 59 and 65 μU per ml which should be compared with the mean of 34 μU and a mean range of 27 to 43 μU per ml for normal females less than 50 years of age

The SILA response to oral glucose was studied in seven males and two females with constant glucosuria. Fig. 2 gives the mean and mean range of the SILA values fasting and after 30, 90 and 150 min. The peak at 30 min is slightly lower than that found in the normal controls. There

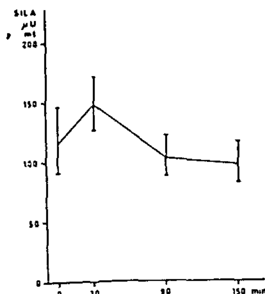



*Fig* SILA (mean and mean range) during oral glucose tolerance test in nine subjects with constant glucosuria.

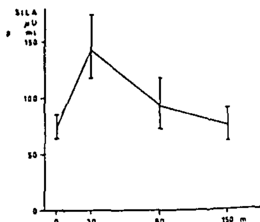



*Fig 3* SILA (mean and mean range) during oral glucose tolerance test in five subjects with constant glucosuria. Normal response

was a great variation in the individual curves Five subjects three males and two females showed a rise at 30 min to a level 50% or more above the fasting value (Fig 3) The remaining four males showed the pattern summarized in Figs 4 *a* and *b* which may suggest a lack of response similar to observations made in patients with diabetes

The subjects with constant renal glucosuria showed a lower per cent body fat (12 6±2 0) than the control group (17 3±1 1) expressed as the mean and S E.M The difference was significant ($p < 0\ 05$)

The fasting SILA in this group showed no correlation to fasting blood glucose age or per cent body fat

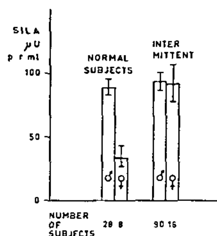
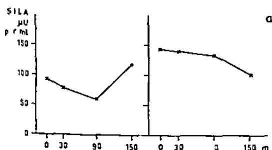



*Fig 5* Fasting SILA in intermittent renal glucosuria



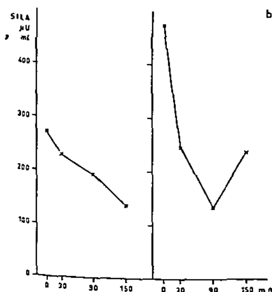




*Fig 4a b* SILA response to oral glucose in four males with constant glucosuria. Diabetic type of curve

## *Intermittent glucosuria*

Fasting SILA determinations were performed in 90 males and 16 females In the male group 36 were less than 50 years of age and 54 more than 50 years of age (18–83) (total range) All the females except one were less than 50 median age 32 (19–54)

The results are presented in Fig 5 The mean values for males was 94 (81–101) μU per ml—mean and mean range There was no difference in the corresponding SILA in the control group—89 (83–96) μU per ml

The SILA in the female group was 92 (78–107) μU per ml which was significantly increased ($p < 0\ 001$) above the values for the control group —34 (27–43) μU per ml

The SILA response to oral glucose was studied in 20 males selected at random from the group with renal glucosuria of the intermittent type (Fig 6) The peak at 30 min was slightly lower than in the normal group but not at a significant level and at 90 min the decrease was less than in the normal group When the individual curves were analysed five were found to differ considerably from the remaining 15 (Fig 7) There was practically no response at 30 and 90 min This type of curve has only been seen in subjects with diabetes Two other subjects showed a rise of only 20 and 25% above the fasting level

The group with intermittent glucosuria showed no correlation between fasting SILA values and fasting blood glucose or between SILA and age or body weight or per cent body fat

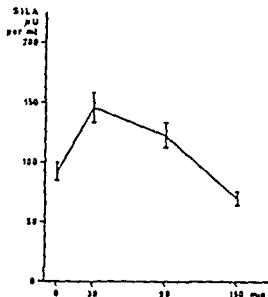


Fig 6 SILA (mean and mean range) during oral glucose tolerance test in twenty males with intermittent renal glucosuria.

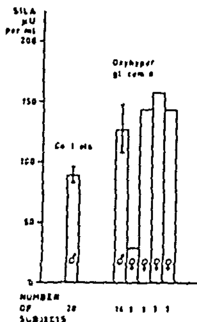


Fig 8 Fasting SILA (mean and mean range) in fourteen males with oxyhyperglucemia.

*"Oxyhyperglucemia*

Fasting SILA determinations were performed in 14 males with a median age of 55 years (21 to 65 years) and four females 23 49 69 and 72 old As Fig. 8 shows the mean fasting A for the male group was 127 (109–148) μU ml This was significantly increased above the SILA values for the control group ($p < 0.05$) In the female group one 23 year-old girl had a value of 29 which is within the normal range for her age group The remaining three had SILA levels of 144 158 and 144 μU per ml which is above the normal range of 67–83 μU for women more than 50 years of age

Thus with one exception all subjects with "oxyhyperglucemia" had increased fasting SILA values. There was no correlation to fasting blood glucose age body weight or per cent body fat. SILA determinations during the oral glucose tolerance test were performed in 5 of the 14 males with "oxyhyperglucemia" (Fig 9) The peak reached after 30 min and the decrease after 90 min corresponded to the pattern observed in the normal group

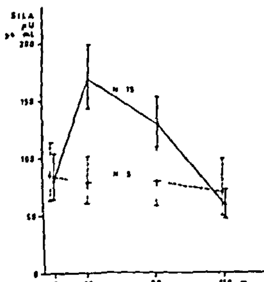


Fig 7 SILA (mean and mean range) during oral glucose tolerance test in twenty males with intermittent renal glucosuria. Two patterns of response

## DISCUSSION

The present study is concerned with non-diabetic glucosuria. Two types of non-diabetic glu osuria can be recognized *renal glucosuria* with a com pletely normal blood glucose curve following an oral dose of glucose and *oxyhyperglucemic* non diabetic glucosuria with a very fast rise in blood glucose above the normal during the first hour and thereafter normalization of the level From a practical point of view it might be important to

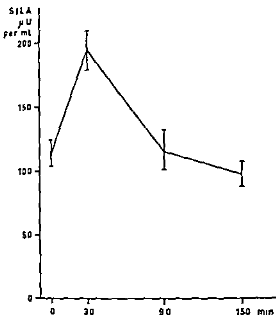



*Fig 9* SILA (mean and mean range) during oral glucose tolerance test in five males with oxyhyperglucemia.

identify non-diabetic glucosuria in an individual so as to avoid unnecessary future investigations when the patient is taken ill with other disabilities

It is obviously necessary to demonstrate that the finding of glucose in the urine is not a sign of very early diabetes Hales et al (5) studied a group of subjects with normal glucose tolerance and with coincident glucosuria They found that the mean plasma insulin determined by immuno assay was higher than normal 30 and 60 min after an oral dose of glucose They also measured plasma growth hormone and found normal values fasting and after glucose An increased basal out put of insulin was thought to be a contributing factor to the poor insulin response in diabetes Therefore this group with normal glucose toler ance could still represent subjects prone to develop true diabetes at a later stage

Many reasons thus motivated a study of sub jects who presented with glucosuria during the diabetes campaign but showed no indication of diabetes in the oral glucose tolerance test We have determined the serum insulin like activity by the epididymal fat pad technique in order if possible to obtain information about the insulin glucose relation from a biological point of view Most recent studies of serum insulin make use of immunoassays (5) and obtain information con cerning the presence of insulin as a protein A comparative study of these techniques has recently been published by Kajinuma et al (6) In serum fractionated by gel filtration two fractions were obtained which had SILA activity in the fat pad assay but only one of them contained the im munoreactive insulin

Lundquist (8) observed that the insulin effect in the fat pad assay was suppressed by cortisol added in vitro In subjects with newly discovered diabetes high fasting levels of cortisol were found (9) It has not yet been determined whether a high cortisol level in plasma can in vivo inhibit the effect of insulin We too have therefore felt justi fied in using the fat pad technique in the present studies as information may be obtained that is not accessible by the immunoassay techniques

Non-diabetic glucosuria of the renal type may be present constantly or only intermittently In the present study these groups were treated sepa rately

Male subjects with *intermittent renal* glucosuria showed normal SILA in the fasting state After oral glucose intake five out of 20 subjects in this group showed practically no SILA response which suggested that they may be candidates for the later development of clinical diabetes (4 15) The remaining 15 showed a normal SILA response to glucose

Female subjects with intermittent glucosuria showed in all 16 cases fasting SILA values significantly above the normal control group This may indicate an increased basal output of insulin although the power of responding to glucose has not so far been exhausted as suggested by Hales et al (5) Such a possibility would make it diffi cult to exclude a risk of developing diabetes also for this group

*Constant renal* glucosuria in male subjects was accompanied by normal fasting SILA but follow ing oral intake of glucose four out of nine subjects showed *no* SILA response Constant renal gluco-suria was thus accompanied by a diabetic type SILA response to glucose in nearly half of those studied and the same pattern was noted in one fourth of those with intermittent renal glucosuria The figures are too small to permit the conclusion that constant glucosuria represents a more severe condition i e more related to diabetes than inter mittent renal glucosuria. Further studies are indi cated

In subjects with *oxyhyperglucemia* the fasting SILA in 14 males was significantly increased above the values in the normal control group. If an increased basal output of insulin carries with it a risk of exhaustion, then also a state of oxyhyperglucemia should represent an increased risk of developing diabetes. The SILA values during the oral glucose loading may actually be lower than expected from the high blood glucose levels, i.e. *the insulinogenic index* (14) was 0.50, which should be compared with the insulinogenic index for the diabetic group, 0.14, and 1.22 for the normal controls (3).

The impression gained from these studies is that even a postprandial positive Clinistix test may indicate a deficiency in the insulin response, although the oral tolerance test judged by our criteria is completely normal.

This focuses attention on the reliability of the Clinistix technique for detecting glucose in the urine. Those we have studied were all positive, but were any missed? According to studies by Schersten (11) the outcome is not only determined by the amount of glucose present, but to a great extent by the presence of substances which inhibit the glucose oxidase reaction. The most important inhibitory substance appears to be ascorbic acid. Schersten found in in vitro studies that the dark color reaction given by 500 mg of glucose per 100 ml was completely inhibited by 50 mg of ascorbic acid per 100 ml. When 20 mg of ascorbic acid per 100 ml was present, no color reaction was given by the Clinistix test paper to 100 mg of glucose per 100 ml. Normally glucose is present in the fasting state in the urine in amounts between 2 and 20 mg per 100 ml (12). A glucose concentration of 100 mg per 100 ml in the urine is therefore pathological. Ascorbic acid concentrations in the urine exceeding 10 mg per 100 ml are frequently seen (13). It could very well be that the findings during the Bedford study (2) of many persons with no glucosuria but hyperglucemia were due to some extra orange juice taken with the Sunday breakfast, after which the urine was collected. Considerable uncertainty may thus be involved in studies based on the Clinistix test. This has a bearing also on our findings. We have no precise information about the prevalence of non-diabetic glucosuria in the population. We can only assume that those with a positive test had, in proportion to the amount of glucose present, a low level of inhibitors. It is obvious that techniques which eliminate the effect of enzyme inhibitors when measuring glucose in the urine are highly desirable.

The poor SILA response in several subjects with glucosuria and non-diabetic glucose tolerance in the present studies should be compared with our normal material based only on the normal glucose tolerance test and the absence of glucosuria, in which no subject with a similar poor response in the SILA determinations was found. Furthermore, a group of subjects with borderline glucose tolerance, "between 2 and 3 S.D.", has also shown a rise of SILA in the normal range. The borderline series, which is still under study, has been followed for 3 to 5 years with no signs of a deteriorating glucose tolerance (3). A normal glucose tolerance test in the presence of a positive Clinistix test may, according to our findings, be more related to a diabetic insulin response than a glucose tolerance curve with blood sugar "between 2 and 3 S.D.".

## REFERENCES


1. Brandt, L., Nordén, A., Scherstén, B. & Tryding, N.: A diabetes detection campaign in Southern Sweden. Results of 69 000 examinations. Acta med. scand. 176: 555, 1964.
2. Butterfield, W. J. H., Keen, H. & Whichelow, M. J.: Renal glucose threshold variations with age. Brit. med. J. 2: 505, 1967.
3. Carlström, S., Lundquist, A., Lundquist, I., Nordén, A., Scherstén, B. & Wollmark, G.: Studies in subjects with positive postprandial Clinistix® test. III. Special studies with follow up of cases with borderline glucose tolerance. In preparation.
4. Cerasi, E. & Luft, R.: Insulin response to glucose infusion in diabetic and non-diabetic monozygotic twin pairs. Genetic control of insulin response. Acta endocr. (Kbh.) 55: 330, 1967.
5. Hales, C. N., Greenwood, F. G., Mitchell, F. L. & Strauss, W. T.: Blood glucose, plasma insulin and growth hormone concentrations of individuals with minor abnormalities of glucose tolerance. Diabetologia 4: 73, 1968.
6. Kajinuma, H., Ide, T., Kuzuya, T. & Kosaka, K.: Insulin-like activity by fat pad assay and immunoreactive insulin. II. Their occurrence in fractions by gel filtration of serum. Diabetes 18: 75, 1969.
7. Lawrence, R. D.: Symptomless glycosurias: differentiation by sugar tolerance tests. Med. Clin. N. Amer. 31: 89, 1947.
8. Lundquist, I.: On the significance of serum dilution and cortisol antagonism in the rat fat pad bioassay of insulin. Acta endocr. 58: 11, 1968.

9 Lundquist, I Norden, A & Schersten B Studies in subjects with positive postprandial Clinistix® test. I Serum insulin like activity (SILA) and free cortisol in newly discovered diabetics Acta med. scand. 187 163 1970

10 Marble A.. Renal glucosuria. Amer med Sci 183 811 1932.

11 Schersten, B Critical analysis of urinary glucose measurements in diabetes detection and diagnosis. In Scandinavian Diabetic Symposium on Control in Diabetes, Hook, Sweden 1968 (ed S E. Fagerberg & J Östman) pp 80-85 C Hamburgers Bogtrykkeri A S Copenhagen 1968

1 Schersten, B & Fritz, H Subnormal levels of glucose in urine A sign of urinary tract infection J Amer med Ass. 201 949 1967

13 Schersten, B & Thysell, H Unpublished observations

14 Seltzer H S Allen E W Herron, A. L. Jr & Brennan M T Insulin secretion in response to glycemic stimulus relation of delayed initial release to carbohydrate intolerance in mild diabetes J clin Invest 46 323 1967

15 Yalow R. S & Berson, S A. Plasma insulin concentrations in non-diabetic and early diabetic subjects Diabetes 9 254 1960

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# A SMALL OUTBREAK OF COXSACKIE B5 INFECTION WITH TWO CASES OF CARDIAC INVOLVEMENT AND ORCHITIS FOLLOWED BY TESTICULAR ATROPHY

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*Abstract* An outbreak of Coxsackie B5 infection in a group of six male students is reported Four of the patients had the symptoms of an uncomplicated enterovirus infection with fever chills muscular pains etc In two cases there was, however marked cardiac involvement with severe chest pains and ECG changes In addition these two patients had orchitis with secondary testicular atrophy

Immunoglobulin separation and demonstration of IgM antibodies against Coxsackie B5 was used for the confirmation of the virological diagnosis in three of the cases The value of this technique is discussed in the paper

The clinical manifestations of infections with Coxsackie B viruses are varying Several reports have described cardiac involvement and affections of the testes have also been reported As cardiac and testicular involvement often are late symptoms of Coxsackie infection a laboratory diagnosis of the infection cannot always be achieved by means of virus isolation or conventional serological technique

The present report deals with six patients with Coxsackie B5 infection two of whom suffered from chest pains and orchitis

## MATERIAL

The patients were all male students four of whom studied medicine On Dec 13 1968 these students joined each other for a dinner party Our interest was aroused when two of the patients developed the unusual combination of cardiac symptoms and orchitis. Some of the patients were not seen until after having recovered which explains the lack of virological examinations in the acute phase in some of the cases

## METHODS

*Isolation of virus*

Stool specimens were collected from three of the patients and inoculated into cultures of GMK and HeLa cells as well as in suckling mice by the intracerebral and subcutaneous routes. The cell cultures were observed every second day for the presence of cytopathic effect (CPE) If no CPE was observed on the sixth day a blind passage was made The mice were observed 11 days for signs of paralyses

*Complement fixation tests (CF tests)*

Sera inactivated at 56 C for 30 min were tested in dilutions ranging from 1 4 to 1 256 against antigens derived from Coxsackie virus type B5 adenovirus mumps virus Mycoplasma pneumoniae and Toxoplasma gondii Antigen and serum controls as well as complement titrations were included in all tests

*Neutralization tests (NT)*

Inactivated sera were tested before and after separation of IgM and IgG antibodies The immunoglobulin separations were performed by gel filtration on Sephadex G 200 (AB Pharmacia Uppsala, Sweden) All sera or fractions were diluted 1 2 to 1 64 and mixed with 100 $TCID_{50}$ of Coxsackie virus type B5 before inoculation into GMK cultures As a control a virus titration was included in the tests

*Antibody titres*

All antibody titres are given as reciprocal values of the highest serum dilutions showing antibody activities

## CASE REPORTS

*Case I*

On Dec 11 1968 this 29 year-old man fell ill with fever His temperature rose to 39 C and lasted until Dec 13 when he took part in the dinner party mentioned above. On Dec 14 he felt well again but twice during the

| Orchitis | Epididymitis | Pericardial friction rubs | ECG changes | Abnormal chest X ray |
|---|---|---|---|---|
| – | – | NE | NE | NE |
| – | – | + ( ) | – | – |
| – | – | NE | NE | NE |
| – | – | – | – | – |
| + | + | + | + | – |
| + | – | – | – | – |

was located in the forehead and vertex. The following day he experienced muscular pains in his back, arms and legs, as well as retrobulbar pains. Also eye movements were painful. These symptoms lasted until Dec. 19 but were replaced by severe pains on the left side of his chest, accentuated by inspiration. He also felt nausea and had slight diarrhoea. The chest pains increased during the following days and caused him to seek medical aid. On Dec. 21 he was examined by one of the authors (B. O.) at the hospital. Examination revealed a slight pharyngitis and a precordial, systodiastolic friction rub. The ECG showed a slight ST elevation in lead CR4. The chest X ray was normal, the heart size being within normal limits. He was given salicylic acid but the pains did not disappear until Dec. 25. An ECG taken the day before failed to show any ST elevations. On Jan. 2, 1969 he noticed that his left testis and epididymis were tender and slightly swollen. His temperature rose to 40°C. Being away on holiday he was examined by a doctor in another town. The ESR was 26 mm/h and the ECG was still normal. The left testis was approximately 1 / times the normal size. Both the testis and the epididymis were tender when examined. These symptoms and signs disappeared gradually during the following four days. The patient noticed, however that his left testis appeared smaller after some weeks.

*A follow-up examination was made on April 1 1969.* The physical examination was normal except for the left testis, which showed atrophy to a third of its normal size. The ECG and the chest X ray were normal. The size of the heart was the same as before. The patient felt well.

Virus isolation was not attempted at any time. Antibody studies were not made in the acute phase of the illness. Serum taken on Jan. 17 contained CF antibodies in a titre of 32, and neutralizing antibodies in a titre of >64 against Coxsackie B5 in unfractionated serum, 16 in the IgM fraction and <2 in the IgG fraction of the serum. There were no CF antibodies against mumps virus.

*Case 6*

On Dec. 16 this patient, who was 26-year-old, suddenly got severe chills. His temperature rose to 39 C and he experienced back pains and frontal headache. The symptoms diminished gradually and on Dec. 19 he felt well. On Dec. 21 he got severe retrosternal pains accentuated by breathing. On Dec. 24 he felt somewhat better. The same day he was seen at the hospital. An ECG showed low T waves in the chest leads. The patient improved during the following days, but on Dec. 27 the temperature rose again to 38.5 C and remained so until Dec. 31 when he felt well again. On Jan. 3 1969 he noticed that his right epididymis was swollen and tender. Two days later the left epididymis was also affected. The temperature rose again to about 38 C and, due to the fact that he had felt very tired since Jan. 3 he contacted one of the authors (B. O.). On examination he was found to have cyanosis of the lips and dyspnoea on exertion. A third heart sound was heard. The ECG showed inverted T waves over the left ventricle. Both testes and epididymis were swollen and tender especially on the left side. He was hospitalized and treated with bed rest for two days. His temperature was now normal. The ESR was 18 mm/h and the WBC 4600 with a slight lymphocytosis in the differential count (48.5%). Urine analysis showed 10–12 white blood cells per high power field. There was no proteinuria. Serum bilirubin, thymol reaction, alkaline

Table II. *Antibody titres*

Results obtained in complement fixation tests (CF tests) against antigens derived from Coxsackie virus type B5, adenovirus, mumps virus, Mycoplasma pneumoniae and Toxoplasma gondii and in neutralization test (NT) against Coxsackie virus type B5. NT was performed on sera before and after immunoglobulin separation by gel filtration on Sephadex G 200. Antibody titres are given as reciprocal values of the highest serum dilutions showing antibody activities

| Case no | Date of serum | Antibody titre in CF test against | | | | | Antibody titre in NT against Coxsackie B5 | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Coxs B5 | Adeno | Mumps | Mycopl | Toxopl | Whole serum | IgM fraction | IgG fraction |
| 1 | Jan. 10 | <4 | 16 | <4 | <4 | <4 | 32 | N D | N D |
| 2 | Dec. 24 | 8 | <4 | <4 | <4 | <4 | 8 | 2 | 4 |
| | Jan. 14 | 16 | <4 | <4 | <4 | <4 | >64 | N D | N D |
| 3 | Febr 1 | 16 | <4 | <4 | <4 | <4 | >64 | 16 | 32 |
| 4 | Febr 2 | 8 | <4 | <4 | <4 | <4 | >64 | 64 | 8 |
| 5 | Jan 17 | 32 | <4 | <4 | <4 | <4 | >64 | 16 | <2 |
| 6 | Jan 10 | 8 | <4 | <4 | <4 | <4 | >64 | 4 | <2 |

N D = not done

phosphatases and SGOT were normal The chest X ray was normal and an ultrasound examination over the pre cordium did not show any sign of pericardial effusion On his discharge from the hospital on Jan 10 the inverted T waves seen earlier on the ECG had regressed towards the isoelectric line The patient gradually regained his former physical fitness When examined on March 28 his testes were considerably smaller than before the onset of his illness The other physical findings were normal ECG still showed almost isoelectric T waves over the left ventricle His chest X ray was still normal

His case was diagnosed as myocarditis epididymitis and orchitis

From a faecal specimen taken on Jan 9 Coxsackie B5 virus was isolated An attempt to isolate the virus from prostate ecretion was not successful Serum obtained on Jan 10 contained CF antibodies in a titre of 8 and neutralizing antibodies in a titre of >64 against Coxsackie B5 CF test against mumps wa negative

## COMMENTS

Twenty years have gone since the Coxsackie viruses were first recognized It is now well known that they may produce a wide variety of clinical manifestations The flora of symptoms and signs of enterovirus infection has been reviewed by among others Kibrik (9) Thus Coxsackie viruses ave been shown to produce pleurodynia ( Born m disease ) meningoencephalitis paralytic dis perimyocarditis orchitis enteritis pneumo itis pleuritis etc

There are several reports of orchitis and epidi dymitis as complications of epidemic myalgia or Bornholm disease which is now believed to have been a Coxsackie B infection Thus Sylvest (18) reported one case of orchitis among 93 cases of epidemic myalgia Huss (7) found the incidence of orchitis in a Swedish epidemic in 1931 of Bornholm di ease to be 50 out of 884 reported cases In an outbreak of Bornholm disease among British troops in Aden 1946 Jamieson and Prinsley (8) noticed that orchitis was a common com plication developing in 12 out of 30 adult males It occurred in the second or third week of illness Morrison and Baird (10) described a family out break of Bornholm disease in which four out of five ca es were complicated by orchitis

In the Oxford epidemic of Bornholm disease in 1951 proven to be due to a Coxsackie virus Warin et al (19) showed that orchitis developed as a late complication in three out of 30 adult male patients Gordon et al (3) reported one case of orchitis among 60 cases observed in an out break of Coxsackie B5 infection in California in 1956 The combination of pleurodynia myocar ditis and orchitis in a Coxsackie B4 infection was described by Swann in 1959 (17) In 1962 Craig head et al (2) reported a case of orchitis in which Coxsackie B5 virus was isolated from testicular tissue

We think that the closed outbreak of Coxsackie B5 reported in this paper gives a good illustration of the variations in clinical course and severity of this illness

There is good reason to believe that case 1 of the present report was the source of infection The close and long contact between the persons involved probably explains why all of them were infected The incubation time varied between 2 and 4 days These figures are also mentioned in other papers on Coxsackie B epidemics (3 7 17)

Sainani et al (13) and several other authors have pointed out that Coxsackie B infection with heart involvement may result in permanent heart damage Two of our patients had signs of cardiac involvement One of them though clinically in good condition had persisting ECG changes in dicating myocardial damage three months after the initial symptoms

Two of our patients had orchitis and epididy mitis in combination with peri myocarditis In one case only one side was affected and was followed by substantial testis atrophy In the other case testes and epididymis on both sides were affected Bilateral atrophy of both testes was noticed at the follow up examination

In the reports reviewed testis atrophy has not been mentioned as a result of orchitis due to Cox sackie B infection It will naturally be of interest to follow up these two cases with regard to testicular function

The conventional laboratory diagnosis of a virus infection is based upon isolation of virus in speci mens from the patient and/or upon the demon stration of a significant rise in antibody titre in wo sera from the patient With one exception the cases reported in this paper were all investigat ed several weeks after the onset of their symp toms Therefore a conventional diagnosis could be made in only two of the cases in one by means of isolation of Coxsackie B5 from a stool speci men and in another by demonstration of rising titres of neutralizing antibodies against the same virus However all of the patients except one were

found to have complement fixing as well as neutralizing antibodies against Coxsackie B5 in their sera To demonstrate that these antibodies did not derive from other infections some of the sera were fractionated by means of Sephadex G 200 gel filtration and the amount of neutralizing antibodies against Coxsackie B5 in the IgM and IgG fractions was investigated By means of this technique IgM antibodies could be demonstrated in all sera As IgM antibodies normally disappear about eight weeks after the onset of an infection this finding strongly indicated a connection between the antibody titres in the sera from the patients and their symptoms

*Patients with cardiac disease of viral aetiology* are often admitted to hospital rather late after the beginning of their infection The virological diagnosis will therefore in many cases be restricted to the demonstration of high antibody titres in single sera The fractionation of sera in IgM and IgG antibodies or the reduction of IgM antibodies with 2 mercaptoethanol (4) will then open a way for the confirmation of a suspected diagnosis

## REFERENCES


1 Bain H W McLean D M & Walker S J Epidemic pleurodyna (Bornholm disease) due to Coxsackie B5 virus. Pediatrics 27 889 1961

2 Craighead E Mahoney E M Carver D H Naficy K & Fremont-Smith P Orchitis due to Coxsackie virus group B type 5 New Engl J Med 261 937 1959

3 Gordon R B *Lenette* E H & *Sandroch* R. S The varied clinical manifestations of Coxsackie virus infections Arch intern. med. 103 63 1959

4 Hanson, L. A Lundgren S Lycke E Strannegård Ö & Winberg J Clinical and serological observations in cases of Coxsackie B3 infections in early infancy Acta paed at scand 55 577 1966

5 *Hedlund, P* Lycke E & Tibblin, G The association of acute pericarditis in adults with Coxsackie B-5 virus infection Acta med scand 172 323 1962

6 Helin M Savola, J & Lapinleimu, K Cardiac manifestations during a Coxsackie epidemic Brit Med J 3 97 1968

7 Huss, R. La myalgie épidemique en Suède Bull off int Hyg. publ. 26 1083 1934

8 Jamieson W M & Prinsley D M Bornholm disease in the tropics Brit Med J 2 47 1947

9 Kibrick, S Current status of Coxsackie and ECHO viruses in human disease Progr Med Virol 6 28 1964

10 *Morrison R. J G & Baird J P* Orchitis in Bornholm disease Brit. Med J 1 198 1952

11 Null F C & Castle C H Adult pericarditis and myocarditis due to Coxsackie virus group B type 5 New Engl *J Med 261 937 1959*

12 Pollen, R. H Myocarditis due to Coxsackie virus group B type 5 Amer J Cardiol 12 736 1963

13 Sainani G S Krompotic E. & Slodki, S J Adult heart disease due to Coxsackie virus B infection Medicine 47 133 1968

14 Sanders V Viral myocarditis (editorial) Amer Heart J 66 707 1963

15 Smith W G Coxsackie heart disease in adults (editorial) Amer Heart J 73 439 1967

16 — Adult heart disease due to Coxsackie virus group B Brit. Heart J 8 04 1966

17 Swann N T Epidemic pleurodynia orchitis and myocarditis in an adult due to Coxsackie virus group B type 4 Ann intern Med 54 1008 1961

18 Sylvest E Epidemic myalgia Oxford University Press London 1934

19 Warin J F Davies J B M Sanders, F K & Vizoso A D Oxford epidemic of Bornholm disease *1951 Brit Med. J 1 1345 1953*

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# EXTRACORPOREAL IRRADIATION OF THE BLOOD AS IMMUNOSUPPRESSIVE TREATMENT IN RENAL TRANSPLANTATION

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*Abstract* Seventeen consecutive patients in terminal uraemia were treated with extracorporeal irradiation of the blood (ECI) as pretransplantation immunosuppressive therapy Eleven patients have received a kidney transplant, and eight of these were treated with ECI after the transplantation too

The transit dose employed was 280–610 rads the average total dose 54 600 rads. In all patients the lymphocyte concentration in the blood decreased during ECI on the average to 0% of pretreatment values, or to 290 ± 110/μl No correlation was observed between transit dose and decrease in lymphocyte concentration.

After ECI the lymphocyte counts have remained below 50% of pretreatment values in all patients including those who were not transplanted and therefore did not receive other immunosuppressive therapy (maximum 14 months of observation)

Ten patients received a kidney transplant from a related living donor (2 A1 match 6 C match 2 D match) and one a necrokidney (D or E match) All transplanted patients were treated with azathioprine and prednisone the dosages employed were relatively small Two rejection episodes have occurred both slight and reversible and renal function is now 1 to 12 months after transplantation normal in all

The importance of the small lymphocyte in the rejection of allografts has been clearly demonstrated (9) Both in the afferent (18) and in the efferent (7) part of the immune response to foreign transplantation antigens the small lymphocytes play a decisive role A large proportion of these cells have a long life span continuously circulating between blood and lymphoid tissues Furthermore the small lymphocyte is the most radiosensitive cell in the blood These findings form the basis for the use of extracorporeal irradiation of the blood (ECI) in order to deplete the organism of small lymphocytes and thereby weaken the immune reaction against allografts

In Rigshospitalet ECI is employed for pretreatment of recipients of renal allografts and in this article fifteen months experience is presented

## MATERIAL AND METHODS

Between February 1968 and April 1969 17 consecutive patients in terminal uraemia were treated with ECI before kidney transplantation (Table I) All patients were haemodialyzed twice a week, thus maintaining an average serum creatinine concentration of 6–8 mg/100 ml Haemodialysis was carried out via a Scribner shunt in 12 of the patients in the remaining five via a subcutaneous arteriovenous fistula (3) In two of the latter patients the fistula had to be changed to a Scribner shunt because the daily use of the fistula for ECI treatment had impaired its function

The apparatus for extracorporeal irradiation consisted of a Co source of approximately 600 Ci, surrounded by a stationary lead shielding (1) During treatment blood was running through a plastic tubing past the source and back to the patient Treatment was carried out 8–10 hours per day and 3–5 days per week A mean cumulative erythrocyte dose (13) of at least 40 000 rads was aimed at, resulting in a treatment period of 10– 0 days (74–174 hours) In three patients two such series were given at intervals of 2–4 months

The blood flow through the irradiation field was 55–120 ml/min average 75 ml/min In order to obtain a satisfactory flow rate a pump (Travenol) was often used Dosimetry was performed by the ferrous sulphate method (8) and the radiation dose was calculated as described by Marsaglia and Thomas (13) The average transit dose was 280–610 rads (Table I) with a mean of 435 rads The total dose given was 32,100–72 800 rads, mean 54 600 rads

During ECI treatment all patients were anticoagulated with heparin 5000–7500 IU at start, followed by 1500 IU approximately every hour guided by the coagulation time which was maintained above 60 min.

Leukocyte and differential counts were performed daily determinations of haemoglobin and thrombocyte concentrations twice weekly

Table I *Data on 17 uraemic patients treated with extracorporeal irradiation*

The lymphocyte concentration given is the average of three determinations

| | | | | ECI | | | | Lymphocyte concentration µl blood | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | At the end of ECI | |
| Pat no | Sex | Age (y) | Duration of uraemia (y) | Duration (h) | Average blood flow (ml/min) | Average transit dose (rads) | Mean cumulative erythrocyte dose (rads) | Before ECI | 1st series | 2nd series |
| 1 | ♀ | 44 | 4 | 96 | 55 | 610 | 45 000 | 1100 | 100 | |
| 2 | o | 27 | 3 | 88 | 70 | 520 | 43 800 | 2000 | 400 | 300 |
| 3 | ♂ | 33 | 2 | 129 | 65 | 505 | 43 800 | 1700 | 300 | |
| 4 | o | 35 | 2 | 133 | 70 | 465 | 55 500 | 1[illegible]00 | 300 | |
| 5 | ♂ | 25 | 4 | 117 | 100 | 340 | 69 700 | 800 | [illegible]00 | |
| 6 | o | 50 | 4 | 141 | 95 | 345 | 65 600 | 1300 | 400 | 400 |
| 7 | ♂ | 44 | 2 | 121 | 90 | 350 | 63 500 | 2700 | 300 | |
| 8 | o | 41 | 2 | 125 | 65 | 465 | 57 800 | 1200 | 400 | |
| 9 | ♀ | 33 | 4 | 140 | 80 | 375 | 72 800 | 1800 | [illegible]00 | |
| 10 | o | 44 | 4 | 131 | 70 | 440 | 53 300 | 1700 | [illegible]00 | |
| 11 | ♂ | 51 | 5 | 130 | 70 | 500 | 62 000 | 1400 | 300 | |
| 12 | ♂ | 43 | 4 | 134 | 70 | 450 | 45 700 | 1100 | [illegible]00 | |
| 13 | o | 24 | 3 | 115 | 120 | 280 | 50 600 | 1[illegible]00 | 400 | |
| 14 | ♀ | 23 | 5 | 101 | 75 | 445 | 57 100 | [illegible]000 | [illegible]00 | 100 |
| 15 | o | 59 | 3 | 174 | 70 | 405 | 68 100 | 1600 | 300 | |
| 16 | ♀ | 31 | 10 | 98 | 70 | 445 | 42 900 | 1400 | 300 | |
| 17 | ♂ | 43 | 10 | 74 | 65 | 445 | 32 100 | 700 | 300 | |

Eleven of the 17 patients treated with ECI have received a kidney transplant. In ten a close relative (2 8 parents) volunteered as donor In two cases typing revealed identical transplantation antigens in donor and recipient (A1 match) in six cases the [illegible]or possessed one transplantation antigen (C match) and in two cases two transplantation antigens (D match) which the recipient did not possess (11) In these ten patients ECI treatment was given shortly before transplantation Patient no 6 received a necrokidney in this case the donor possessed two or three transplantation antigens which were not found in the recipient (D or E match) This patient received two series of ECI, the last 2 months before transplantation

In the remaining six patients transplantation from a living (related) donor has not been possible and in these patients the time for ECI treatment was chosen arbitrarily They are now waiting for transplantation of a necrokidney

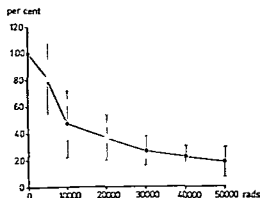



*Fig 1* Lymphocyte concentration in the blood during extracorporeal irradiation. Data from 16 patients. (One patient (no 4) is excluded because of only one pretreatment value of 800 lymphocytes µl.) Abscissa. mean cumulative erythrocyte dose Ordinate lymphocyte count in per cent of pretreatment value average values ± S.D

## RESULTS

The effect of ECI treatment must at present be judged from two parameters

1 Decrease of the lymphocyte concentration in the blood during treatment and duration of the lymphocytopenia.
2 *Clinical course after renal transplantation*

### *1 Lymphocytopenia*

In Table I the lymphocyte concentration in the blood before and after treatment is shown (average of at least 3 days) The values observed before ECI were low (mean 1460 ± 480 lymphocytes/µl) in accordance with previous findings in uraemic patients (19) After treatment the mean concentration was 290 ± 110/µl or one fifth of pretreatment values Fig. 1 shows that at *10 000* rads the lymphocyte concentration was reduced to approximately one half of pretreatment values,

at 30 000 rads to one fourth During continued treatment the rate of decline decreased and at 50 000 rads the lymphocyte concentration was on the average 19 % of pretreatment values No correlation was observed between decrease in lymphocyte concentration and average transit dose

During the period of extracorporeal irradiation the concentration of neutrophils and of thrombocytes was unchanged in 14 patients In three the neutrophils decreased by 50 % on the average The requirements for blood transfusions seemed to increase slightly This can be explained by the more frequent blood sampling and by the radiation induced haemolysis which is seen when the mean cumulative erythrocyte dose exceeds approximately 25 000 rads (2)

Fig. 2 shows the lymphocyte concentration in the blood after cessation of ECI in those patients who did not receive a transplant immediately after ECI treatment. It appears that the average lymphocyte concentration has remained below 50 % of the pretreatment value one patient (no 11) has been observed for more than one year and his lymphocyte count is still only one third of the pretreatment value

All the transplanted patients received other immunosuppressive treatment and their lymphocyte concentration has remained below 500/μl

*Clinical course*

The clinical course of the 11 patients who received a kidney transplant is shown in Table II

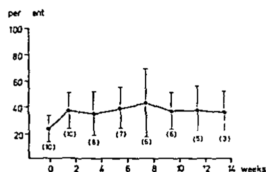



*Fig 2* Lymphocyte concentration in the blood after cessation of extracorporeal irradiation (ECI) in patients who did not receive further immunosuppressive treatment Abscissa weeks after last ECI treatment. Ordinate lymphocyte count in per cent of pretreatment value average values ± S.D Number of patients in parentheses.

The serum creatinine concentration became normal within 1–2 days in five patients within 1–2 weeks in a further four in the last two patients serum creatinine became normal after 3 weeks The cause of the slow decrease was in five of the six patients reversible tubular damage as judged from the course and (in three cases) from the histological findings in a biopsy specimen acute tubulo-interstitial nephropathy was the most likely diagnosis In the last patient the slow decrease was due to rejection (see below) Renal function judged by serum creatinine concentration or creatinine clearance is now normal in all patients and proteinuria is absent (Table II)

The immunosuppressive regimen after trans-

Table II *Data on the eleven transplanted patients*

| Pat. no | Donor | Match | Normal serum creat nine days after transplantation | Rejection episodes | Status May 1 1969: Time elapsed since renal transplantation days | Serum creatinine (mg/100 ml) | GFR (ml min) | Proteinuria | Blood pressure (mm Hg) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Father | D | 1 5 | 0 | 349 | 1.2 | 65 | 0 | 130 90 |
| 2 | Father | C | 24 | 1 | 254 | 1 5 | 55 | 0 | 130 80 |
| 3 | Sister | A | 15 | 0 | 227 | [illegible].0 | 65 | 0 | 150 100 |
| 4 | Mother | C | 10 | (1) | 213 | 1 4 | 55 | 0 | 140 90 |
| 5 | Mother | C | 1 5 | 0 | 169 | 1 1 | 90 | 0 | 140 95 |
| 6 | Unrelated | D or E | 2 | 0 | 141 | 1 0 | 60 | 0 | 1 0 90 |
| 7 | Sister | A | 1 5 | 0 | 113 | 0 9 | 80 | 0 | 1 0 90 |
| 8 | Mother | C | 1 5 | 0 | 85 | 1 5 | 65 | 0 | 135 90 |
| 9 | Mother | C | 5 | 0 | 64 | 1 4 | 50 | 0 | 150 110 |
| 10 | Mother | C | 21 | 0 | 42 | 1 4 | 65 | 0 | 135 95 |
| 14 | Mother | D | 10 | 0 | 21 | 0 9 | 90 | 0 | 1 0 90 |

[a] Glomerular filtration rate measured as the endogenous creatinine clearance

Table III. *The dosages of prednisone and azathioprine (mg/kg body weight) employed following renal transplantation (average values ± S.D.)*

| Months after transplantation | 1 | 2 | 3–4 | 5–6 | 7–8 | 9–10 | 11–12 |
|---|---|---|---|---|---|---|---|
| Number of patients | 11 | 10 | 8 | 6 | 5 | 1 | 1 |
| *Azathioprine (mg/kg body weight) (mean and S.D.)* | 2.3 ±0.7 | 1.9 ±0.5 | 1.8 ±0.5 | 1.6 ±0.5 | 1.8 ±0.4 | 1.7 | 1.7 |
| Prednisone (mg/kg body weight) (mean and S.D.) | 0.7 ±0.4 | 0.6 ±0.3 | 0.5 ±0.3 | 0.3 ±0.1 | 0.2 ±0.1 | 0.1 | 0.1 |

plantation consisted of azathioprine, prednisone and ECI. Azathioprine was begun 3 days before transplantation, dosage 3 mg/kg for two days and then 5 mg/kg, since no treatment was given on the day of transplantation. After the operation a maintenance dose of 1.5–3 mg/kg was given daily (Table III); more than 3 mg/kg was never given. Only in one patient was granulocytopenia observed (minimum 300 neutrophils/$\mu$l) and it disappeared after treatment had been stopped for two weeks.

Prednisone therapy was started routinely 3–4 days after transplantation. The average doses during the months following transplantation are given [in T]able III. Seven patients received maximally [...]g/day. Four patients received higher doses [...] 150 mg/day, rapidly tapered off to maintenance doses of 15–30 mg/day. In two of these cases high doses of prednisone were given because of fever within the first two days after transplantation without demonstrable infection. Both patients (nos. 1 and 6) had received their transplants from donors characterized as D matches, and in both renal function was normal on the second day after transplantation (Table II). In one patient a large steroid dosage was employed from day 10 after transplantation because of a rise in serum creatinine after an initial fall. During the vigorous prednisone treatment serum creatinine became normal within a few days, suggesting a rejection episode. In this case the donor was characterized as a C match. The last of these four patients, also a C match, showed 1½ months after transplantation a slight increase in serum creatinine and proteinuria, both disappearing rapidly after increase of steroid dosage, possibly indicating a slight rejection episode.

The steroid treatment has on the whole been well tolerated. Two of the four patients who received larger doses have had complications (in one a bleeding duodenal ulcer and pulmonary tuberculosis, in the other meningoencephalitis due to leptospirosis); none of these were fatal.

After transplantation eight of the recipients have been treated with ECI for 2–54 hours. Due to a marked tendency to clotting of shunt or fistula immediately after transplantation (16) this postoperative ECI treatment could not be carried through with any consistency.

Only one patient (no. 14) received X irradiation of the transplant (150 rads), and none were treated with actinomycin C.

## DISCUSSION

The basic animal studies on extracorporeal irradiation of the blood (ECIB) and of thoracic duct lymph (ECIL) have been carried out by Cronkite's group (5, 6, 10). It was shown that following ECIB not only the blood but also thoracic duct lymph, lymph nodes and spleen were depleted of small lymphocytes. Intermittent ECIB before and after skin transplantation increased the survival time of the transplant, and a further increase was observed if together with ECIB azathioprine was given in doses too small to be effective by themselves. Experiments with ECIL have further demonstrated the importance of the small lymphocyte in the immune response. The thoracic duct of the experimental animals was cannulated so that continuous irradiation of the lymph could be carried out. Thereafter skin grafts from two donors were placed on the ECIL-treated animals, from one donor on the right side of the withers (anterior grafts), from the other in the area near the iliac crest (posterior grafts). The posterior grafts were within the drainage bed of the thoracic duct, whereas the anterior grafts were not drained

exclusively by the thoracic duct Survival time for the anterior grafts was moderately prolonged while the posterior grafts survived as long as con tinuous ECIL was maintained The most reason able explanation of these findings is that rejection of the anterior grafts was prolonged because of the lymphocyte depletion induced by ECIL, where as the permanent survival of the posterior grafts was due to effective blockade of the immunization arc through destruction of the lymphocytes leav ing the regional lymph nodes via the thoracic duct.

The survival of skin grafts in baboons was considerably prolonged if ECIB treatment was given *before transplantation (17)* The lymphoid tissues showed severe depletion of lymphocytes after ECIB

In renal transplantation the small lymphocytes of the blood are decisive in the sensitization of the recipient (18) and a reduction of their number through ECI therefore seems rational In dogs Wolf et al (20) have found a moderate prolonga tion of kidney graft survival after pretreatment of the animals with ECI

In *human organ transplantation the experience* with ECI is still limited In rejection crises *after* renal transplantation ECI has been attempted by several groups (4 12 14 20) in some cases with favourable results

As *immunosuppressive treatment before renal* transplantation the only systematic investigations are those of Persson and collaborators (15) who found significantly fewer rejection episodes in 18 ECI pretreated patients as compared to 60 patients who did *not receive ECI* The dosage of aza thioprine given after transplantation was smaller in the ECI pretreated group the difference was not, however statistically significant. The average total radiation dose was 26 000 rads and a 50 % reduction of the *lymphocyte concentration in* the blood was obtained

In all 17 patients of the present material a pronounced lymphocytopenia was induced by ECI The decrease in lymphocyte concentration contin ued as long as ECI was *given. After cessation of* treatment the lymphocyte count remained low possibly indicating that long term ECI treatment depletes the lymphoid tissues also in humans

In all 11 transplanted patients the clinical course was benign without irreversible rejection episodes the dosages of immunosuppressive drug therapy employed were small To which degree these grati fying results are due to the ECI treatment given is at present difficult to evaluate and the most urgent task is now to define which groups among kidney recipients are benefited by ECI A main advantage of ECI is that it is possible to employ this immunosuppressive treatment before trans plantation without endangering the postoperative course

## ACKNOWLEDGEMENTS

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## REFERENCES

1 *Andersen, V Sørensen S F & Killmann S Aa.* Traitement de la leucémie lymphoide chronique par l'irradiation extra-corporelle du sang Nouv Rev *franç* Hemat. 8 709 1968

2 Andersen V Karle H & Dige Petersen H Erythro cyte survival in patients with chronic lymphocytic leukaemia during treatment with extra-corporeal ir radiation of the blood. Scand. J Haemat. In press

3 Brescia, M J Cimino J E Appel K & Hurwich, B J Chronic hemodialysis using venipuncture and a surgically created arteriovenous fistula. New Engl J Med. 275 1089 1966

4 Calne R Y Ref in Cronkite E P & Chanana, A. D Ionizing radiation In Human transplantation (ed. F T Rapaport & J Dausset) Grune & Stratton New York and London 1968

5 Chanana, A. D Brecher G Cronkite E P Joel, D & Schnappauf H The influence of extracorporeal irradiation of the blood and lymph on skin homograft rejection Radiat. Res. 27 330 1966

6 Cottier H., Ruchti C Sordat, B & Cronkite E P Irradiation extra-corporelle du sang circulant moyens d étude des circulation et recirculation lymphocytaires chez le veau Nouv Rev franç Hemat 8 677 1968

7 Elkins, E L Invasion and dest u tion of homologous kidney by locally inoculated lymphoid cells J Exp Med 1 0 39_ 1964

8 Fricke H & Morse S The action of X rays on ferrous sulphate solutions. Phil Mag 7 129 1929

9 Gowans, J L The role of lymphocytes in the destruc tion of homografts Brit med Bull 21 106 1965

10 Joel D D Chanana, A. D Cronkite E P & Schif fer L M Modification of skin allograft immunity by extracorporeal irradiation of lymph Transplantation 5 1192 1967

11 Kissmeyer Nielsen, F & Kjerbye K E Lymphocyto toxic micro-technique Purification of lymphocytes by flotation *Histocompatibility testing, p 381 Munks*gaard, Copenhagen 1967

12 Maginn, R R & Bullimore J A Extracorporeal *irradiation of the blood in renal homograft rejection.* Brit J Radiol 41 127 1968

13 Marsaglia, G & Thomas, E D The radiation dose accumulated by blood during extracorporeal irradiation. Radiat. Res. 25 269 1965
14 Merrill, J P Cronkite E P Schiffer L. & Carpenter C B Extracorporeal irradiation as an adjunct to immunosuppressive therapy Transplantation 4 541 1966
15 Persson, B., Rosengren, B Bergentz, S E & Hood, B Evaluation of preoperative extracorporeal irradiation of the blood in human renal transplantation. Transplantation 7 534 1969
16 Skov P E. & Fjeldborg, O Intermitterende hæmodialyse med brug af intern arterio-venøs fistel Ugeskr Læg. 130 1724 1968
17 Storb R., Ragde H. & Thomas, E. D Extracorporeal irradiation of the blood in baboons. Radiat. Res 38 43 1969
18 Strober S & Gowans, J L The role of lymphocytes in the sensitization of rats to renal homografts. J Exp Med. 122 347 1965
19 Wilson, W E. C., Kirkpatrick, C H. & Talmage D W Suppression of immunologic responsiveness in uremia. Ann. intern. Med. 62 1 1965
20 Wolf J S., Lee, H M., O Foghludha, F T & Hume D M Effect of circulating blood radiation with an extracorporeal strontium[90] shunt on transplantation immunity in dogs and man. Surg. Forum 17 245 1966

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# KIDNEY PRESERVATION WITH HYPOTHERMIA AND HYPERBARIC OXYGEN

## I The Diffusion of Oxygen into the Kidney

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*Abstract* The possibility of supplying kidneys of about the size of normal human kidneys, kept under hypothermia (5 C) and hyperbaric oxygen (15 25 or 50 ATA) with sufficient oxygen when the diffusion of oxygen takes place only from the surface of the organ has been estimated theoretically and experimentally It is concluded that an oxygen pressure of 50 ATA or more is necessary to supply the deepest parts of the kidneys with sufficient oxygen. However the time of ischemia for the deepest parts of the kidneys will be about 10–14 hours when 50 ATA are used. When the oxygen has reached small concentrations in the deepest parts, the outer layers of the kidneys will show high and possible toxic concentrations More over the high oxygen concentrations will be liberated in a gaseous state under decompression and may thus cause further damage to the tissue

Kidney preservation by means of hypothermia and hyperbaric oxygen without continuous perfusion of the kidney has been reported previously (1 2 7 11 12 14 16)

Hypothermia causes a decrease of the oxygen consumption of the kidney (8) The preserving effect of hypothermia on kidneys without blood circulation has been well established (3)

In the publications mentioned hyperbaric oxygen is used to supply the kidneys with oxygen by means of diffusion from the surface of the kidney into the organ Generally it has been found that the combination of hypothermia and hyperbaric oxygen has a better preserving effect than hypothermia alone (1 7 12)

The rate of the diffusion of oxygen into the kidney under varying pressures and consequently the possibilities of supplying the kidneys with sufficient oxygen have however not been clarified either

By means of theoretical reflections and experimental investigations it is the purpose of the present study to estimate the possibility of oxygen supply to kidneys stored at 5 C and under hyperbaric oxygen when the oxygen supply takes place only from the surface of the kidney

In this connection three questions are discussed in the present paper

1 What oxygen pressure must be used to supply kidneys of a certain size with a given diffusion constant and with a known oxygen consumption with sufficient oxygen in all parts of the tissue?

2 How long a time will pass before the oxygen has reached the deepest parts of the kidney?

3 Will the necessary oxygen pressure cause damage to the whole kidney or to parts of it?

1 This question has been considered theoretically by Warburg (18) and Hill (5) The basis of the calculations is the mathematical term for the diffusion rate in isotropic substances which is given by the equation

$$F = -D\frac{dx}{dc} \qquad (1)$$

where $F$ is the rate of transfer per unit area of section $c$ the concentration of diffusing substance $x$ the space coordinate measured normal to the section and $D$ the diffusion constant On the assumption that the oxygen consumption of the tissue is constant and that steady state between the diffusion of oxygen into the tissue and the oxygen consumption of the tissue has been reached Warburg calculated from (1) the so-called "Grenzschnittdicke" The "Grenzschnittdicke" is the layer of tissue which at a certain oxygen

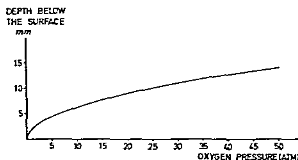



*Fig 1* The curve shows the calculated "Grenzschnittdicke" in kidney tissue when the oxygen pressure at the surface varies between 0 and 50 ATM and the temperature is 5 C

pressure is sufficiently supplied with oxygen and can be calculated by the equation

$$d = \sqrt{8c\frac{D}{A}} \qquad (2)$$

where $d$ is the "Grenzschnittdicke" $D$ the diffusion constant, $A$ the oxygen consumption and $c_0$ the oxygen tension at the surface of the organ.

The diffusion constant for a gas through a substance is defined by Krogh (6) as the number of c c diffusing through 1 cm² and 1 $\mu$ thickness in one minute at a pressure difference of 1 ATM $D$ has been examined experimentally for different tissues by Krogh (6) and Greven (4) Their methods are different, and generally the values of Greven are a little lower than those of Krogh In this work a value of 0 1 cm $\mu$ $min^{-1}$ $atm^{-1}$ has been used

The oxygen consumption of the kidney at 5 C is reduced to about 3% of the consumption at 37 C (8) If that is the case the oxygen consumption at 5 C should be about $20 \times 10^{-4}$ ml/g wet weight min.

If the values for $A$ and $D$ mentioned are used in equation (2) the "Grenzschnittdicke" between 0 and 50 ATA can be calculated (Fig. 1)

In normal-size human kidneys the distance from the surface to the deepest point will be about 15 mm. From Fig 1 it appears that oxygen pressures of 50 ATA or more are necessary to obtain such a "Grenzschnittdicke" when the kidney is stored at 5 C

2 The equation for the "Grenzschnittdicke" can only be applied when steady state between the oxygen consumption of the tissue and the diffusion of oxygen into the tissue has been achieved.

The question of the occurrence of steady state and/or the oxygen concentrations at various depths of the tissue following exposure of the surface of the organ to oxygen are of great importance in kidney preservation since the time of ischemia ought not to exceed a few hours

Hill has calculated equations for determination of the concentrations of oxygen at varying depths of the tissue following exposure of the tissue to oxygen In Fig 2 the calculated values of the oxygen tension in a 15 mm thick kidney tissue are shown for periods varying from 4 to 125 hours after exposure of the tissue to oxygen. For simplicity the oxygen consumption of the tissue has been neglected From Fig. 2 it appears that the entrance of oxygen into the given thickness of tissue is a slow process especially when seen in relation to the desirability of a reasonably short period of ischemia

From the equation of Hill (5)

$$\frac{c}{c_0} = 1 - \frac{4}{\pi} e^{-\frac{K\pi^2 t}{4b^2}} \sin\frac{\pi x}{2b} \qquad (3)$$

where $c_0$ is the concentration of oxygen at the surface $c$ the concentration of oxygen at depth $x$ at time $t$ and $b$ the thickness of the tissue the oxygen tension can be calculated at any point at any time On the assumption that the oxygen consumption is zero it can be calculated that 11 4 hours will pass before the oxygen tension has reached 5 mm Hg at 15 mm depth of the tissue if the pressure at the surface is 50 ATA.

In the above calculations the oxygen consumption of the tissue has been neglected When the

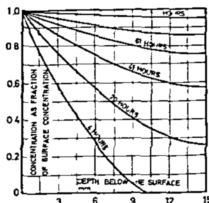



*Fig 2* Each curve shows the distribution of oxygen inside the tissue at a given time (4–125 h) The abscissa represents the depth below the surface of the tissue (0–15 mm), the ordinate the concentration of oxygen as a fraction of that at the surface

oxygen debt and the oxygen consumption of the kidney are taken in consideration it becomes difficult to calculate the rate of entrance of oxygen into the kidney Therefore we have tried experimentally to examine the rate of diffusion of oxygen into kidneys stored under hypothermia (5 C) and with varying oxygen pressures (15 25 or 50 ATA)

## METHOD

Twenty five pig kidneys weighing 65–232 g were used for the study Immediately after removal the kidneys were perfused with cold TIS-U SOL as described by Løkkegaard et al (9) The perfused cooled kidneys were placed on a rack which permitted contact between the oxygen and the whole surface of the kidneys (Fig 3)

A $pO_2$-microelectrode (Beckman microelectrode) suitable for measurement in the tissue was placed at varying depths of the kidneys (Fig 3) Before each experiment the electrode was calibrated with oxygen tensions at 0 and 150 mm Hg at 5 C

The rack with the kidney and the electrode was placed in a pressure chamber which admitted storage under oxygen pressures up to 50 ATA The electrode was connected to a gasmonitor (Radiometer type PHA 927) which was connected to a chart mover The oxygen tension in the kidney was recorded continuously The pressure was raised within 15 min to the oxygen pressure wanted. The whole apparatus was placed in a cold room at 5 C The experimental period varied from 17 to 90 hours Decompression of the pressure chamber took place within 15 min. After removal from the pressure chamber the kidneys were cut along the electrode and the distance from the measuring head to the nearest point on the surface of the kidney was measured

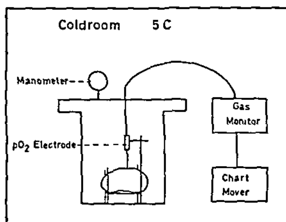



*Fig 3* Schematic representation of experimentel preparation

Table I *Experimental data of 25 $pO_2$ measurements in pig kidneys*

| Kidney no | Oxygen pressure (ATA) | pO -electrode depth below surface (mm) | $pO_2$ at start (mm Hg) | Time from start until $pO_2$ 5 mm Hg (min) | Experimental period (h) |
|---|---|---|---|---|---|
| 32 | 50 | 7 | 0 | 100 | 19 |
| 33 | 50 | 7 | 0 | [illegible] | 22 |
| 34 | 50 | 6 | 0 | 125 | 23 |
| 35 | 50 | 15 | 0 | 840 | 19 |
| 36 | 50 | 14 | 0 | 750 | 26 |
| 37 | 50 | 11 | 0 | 282 | 19 |
| 38 | 50 | 19 | 0 | 1370 | 46 |
| 18 | 25 | 7 | 0 | 360 | 17 |
| 0 | 25 | 4 | 0 | 130 | 19 |
| 21 | 25 | 7 | 0 | 240 | 19 |
| 22 | 25 | 12 | 0 | 665 | 66 |
| 23 | 25 | 2 | 0 | 90 | 18 |
| 25 | 25 | 12 | 0 | [illegible] | 47 |
| 27 | 25 | 14 | 0 | 1565 | 71 |
| 28 | 25 | 18 | 0 | 2960 | 87 |
| 6 | 15 | 10 | 0 | never | 18 |
| 8 | 15 | 7 | 0 | 900 | 17 |
| 10 | 15 | 8 | 0 | 600 | 19 |
| 11 | 15 | 2 | 0 | 95 | 22 |
| 13 | 15 | 3 | 0 | 210 | 24 |
| 19 | 15 | 5 | 0 | 485 | 24 |
| 24 | 15 | 7 | 0 | 915 | 60 |
| 26 | 15 | 12 | 0 | 1860 | 43 |
| 30 | 15 | 15 | 0 | never | 90 |
| 31 | 15 | 12 | 0 | 1710 | 49 |

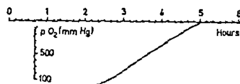



*Fig 4* The pO 4 mm below the surface of the kidney in the first 6 hours after the pressure has been raised to 25 ATA at the surfac of the kidney

## RESULTS

Table I summarizes the most important results After having placed the electrode in the kidney the oxygen tension was found to be zero in all cases The oxygen tension in the kidney tissue began to rise at varying times after the increase of the pressure in the chamber An example is shown in Fig 4 which shows the oxygen tension about 4 mm inside the kidney 0–6 hours after the oxygen pressure was increased to 25 ATA at the surface of the kidney The rate of increase of the oxygen tension and the maximum oxygen tension within the experimental period varied at unchanged pressure with the depth of the elec trode from the surface When the electrode was placed near the theoretical Grenzschnittdicke rate of increase was slow and the maximum oxygen tension low (Fig 5) Near the surface the kidney the oxygen tension rose more rapidly (Fig 4) and the maximum oxygen ten sions rose to high values In one case when the kidney was stored under 15 ATA and the elec trode was placed 15 mm inside the kidney the oxygen tension was found to be 0 for a period of 90 hours (kidney 30 Table I) Fig 6 shows the rate at which the oxygen tension reached 5 mm Hg at varying depths below the surface of the kidney when the pressure at the surface was 15 25 or 50 ATA respectively The time for the oxygen tension to reach 5 mm Hg was chosen because the measurement of this oxygen tension could be carried out with great accuracy In no

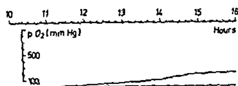



*Fig 5* The pO 12 mm below the surface of the kidney 9–16 hours after the pressure has been raised to 25 ATA at the surface of the kidney

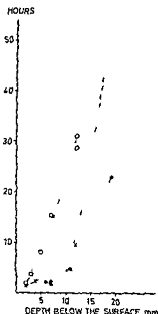



*Fig 6* The three curves show the rate at which the oxygen tension reaches 5 mm Hg in different layers of pig kidneys when the oxygen pressure at the surface is 15 (O--O) 25 (×--×) and 50 ATA (●--●) and the temper ture is 5 C

case did the period from zero to 5 mm exceed 10 min It can be seen that the time of ischemia for the deepest part of a normal size human kidney is probably between 10 and 14 hours when an oxygen pressure of 50 ATA is used

3 The use of high oxygen pressures may damage the tissue because of high oxygen tension in the outer layers of the kidney and because of liberation of physically bound oxygen on de compression of the pressure chamber

Fig 2 shows that the difference in oxygen tensions in the inner and outer parts of the kid ney is very great Using 50 ATA at the surface it can be calculated that the oxyg n tension after 4 hours will be zero at 15 mm but about 20 ATA 5 mm from the surface of the kidney on the as sumption that the oxygen consumption of the kidney is zero

In our experiments it was not possible to meas ure oxygen concentrations above 6000 mm Hg and all measurements above 1500 mm were very inaccurate Yet we were able to establish that high oxygen concentrations were found in the outer parts of the kidneys when 25 and 50 ATA were used In kidney nos 20 23 32 33 and 34

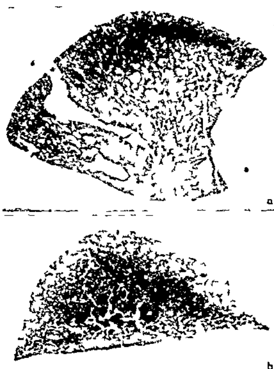



Fig 7 (a) Histological section (×4) of a pig kidney immediately after removal from the pressure chamber after having been stored for 4 hours under 50 ATA oxygen pressure and 5 C Bubbles due to liberation of physically bound oxygen are seen everywhere in the kidney (b) Histological section (×4) of the same pig kidney as shown in a but 6 hours later when the oxygen tension was zero everywhere in the kidney The bubbles which are still seen are probably due to mechanical rupture of the tissu

(Table I) oxygen tensions after 17 hours were found to be about 3500 3800 3500 5000 and 6000 mm Hg respectively In the experiments with 15 ATA no oxygen tensions exceeding 1500 mm Hg were found

After decompression of the pressure chamber microscopical examination of the tissue revealed bubbles in kidneys stored under 15 25 or 50 ATA for 24 hours or more The bubbles were most marked after storage under 50 ATA Fig 7 a shows the kidney just after removal from the pressure chamber and Fig. 7 b the same kidney 6 hours later when the oxygen tension was zero All kidneys stored for 24 hours under 50 ATA were distended and revealed palpable crepitation just after removal from the pressure chamber

## DISCUSSION

Attempts to measure the oxygen tension in kidneys stored with hyperbaric oxygen have been made by Matloff et al (13) Using 4 ATA oxygen pressure they found the oxygen concentrations to be zero in experiments lasting up to 2 hours

Compared with the calculations of Hill where the oxygen consumption has been neglected a reasonable relation to our results is apparent In all cases the time of ischemia in our experiments where an oxyg n consumption of the kidneys must be considered was longer than those calculated from the equation of Hill Yet the comparison must be taken with some reservation The calculations of Hill were carried out for diffusion into plane sheets a condition which was not fulfilled in our experiments

The high oxygen tension in the outer layer of the kidneys when high oxygen pressures are used is of interest in relation to the question of the toxic effect of hyperbaric oxygen (10 15 17)

## ACKNOWLEDGEMENTS

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## REFERENCES

1 Ackermann J R W & Barnard C N Successful storage of kidneys Brit J Surg. 53 5 5 1966
Basso A Calne R Y & Hopkinson W I Renal preservation with hyperbaric oxygenation and hypothermia Brit J Urol 39 2 6 1967
3 Calne R Y Pegg D E Pryse Davies J & Brown F L Renal preservation by ice-cooling Brit med J 2 651 1963
4 Greven K Der O Diffusionskoeffizient von Leber Nierenrinde und Hirnrinde unter verschiedenen Bedingungen Pflugers Arch ges Physiol 71 14 1960
5 Hill A V The diffusion of oxygen and lactic acid through tissues Proc roy Soc B 104 39 19 9
6 Krogh A The rate of diffusion of gases through animal tissues with some remarks on the coefficient of invasion J Physiol (Lond) 57 391 1919
7 Ladaga L G Gault M H Sheldon H & Maclean L D Preservation of the dog kidney J Surg Res 8 495 1968
8 Levy M N Oxygen consumption and blood flow in the hypothermic perfused kidney Amer J Physiol 197 1111 1959
9 Løkkegaard H Fernandes A Gyrd Hansen N Hansen R I Hasselager F Kemp E Lund F & Rasmussen F Kidney preservation with hypothermia and hyperbaric oxygen II Renal clearances in pigs

with autotransplanted 24 hour preserved kidneys Acta med scand 187 195 1970
10 Macdougall J D B & Coupland R F Organ culture under hyperbaric oxygen Exp Cell Res 45 385 1967
11 Makin G S & Howard J M Hyperbaric oxygenation and profound hypothermia to preserve canine kidneys Arch Surg 91 568 1965
12 Manax W G Bloch J H Longerbeam J K & Lillehei R C Successful 24 hour in vitro preservation of canine kidneys by the combined use of hyperbaric oxygenation and hypothermia Surgery 56 275 1964
13 Matloff D B Mobley T L & Schwartz S I Effects of hyperbaric oxygenation on renal ischemia Proceedings of the Third International Conference on Hyperbaric Medicine p 731 National Academy of Sciences National Research Council Washington D C 1966
14 Mirkovitch V Pernard M Hadra S & Hegi F Conservation du rein in vitro sous hypothermie et oxygene hyperbare Schweiz med Wschr 96 1301 1966
15 Popovic V Gerschman R & Gilbert D L Effect of high oxygen pressure on ground squirrels in hypothermia and hibernation Amer J Physiol 206 49 1964
16 Ringdal R Linder E & Pfenninger E Extracorporeal preservation of canine kidney with subsequent reimplantation and contralateral nephrectomy Acta chir scand 33 5 1967
17 Straub J P Mechanism of the toxic effects of hyperbaric oxygen Nature (Lond) 215 1196 1967
Warburg O Versuche an uberlebendem Carcinomgewebe Biochem Z 142 317 1923

Acta med scand Vol 187 pp 195–202 1970

# KIDNEY PRESERVATION WITH HYPOTHERMIA AND HYPERBARIC OXYGEN

## II Renal Clearances in Pigs with Autotransplanted 24 hour Preserved Kidneys

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*Abstract* Preservation of kidneys for 24 hours by means of hypothermia (5 C) and hyperbaric oxygen (50 25 or 15 ATA) has been studied in experiments on pigs.

At none of the oxygen pressures used was survival of the pigs achieved when contralateral nephrectomy was performed in conjunction with transplantation Use of 50 and 25 ATA oxygen pressure caused greater damage to the tissue than 15 ATA. With postponement of contralateral nephrectomy for three weeks after the transplantation, the function of the kidney stored at 15 ATA oxygen pressure was determined by means of inulin endogenous creatinine urea and PAH clearances 10 31 52, 73 and 94 days after contralateral nephrectomy The renal function was reduced in all experiments when compared to a control group of autotransplanted not long term preserved pig kidneys

One of the problems in kidney transplantation particularly where necrokidneys are used is the lack of safe preservation methods In the majority of cases satisfactory preservation for 24 hours will provide sufficient time for thorough tissue typing preparation of the patient and possible transportation of the kidney to the most suitable recipient.

Hyperbaric oxygen in combination with hypothermia without continuous perfusion is one of the simplest methods for such relative short term preservation With the exception of Ladaga et al (5) who used oxygen pressures up to 30 ATA all previous experiments have been carried out with pressures from 2 to 15 ATA for periods of 24–48 hours (1 2 8 9 10 12 13) Due to the failing function of the preserved kidney during the period following transplantation it was necessary to postpone the contralateral nephrectomy by two to four weeks in all the quoted experiments This might be a result of insufficient diffusion of oxygen into the organ at oxygen pressures of 2–15 ATA

It has been found (7) on the basis of theoretical and experimental studies of the diffusion rate of oxygen into kidney tissue that oxygen pressures up to 50 ATA must be used in order to obtain measurable oxygen concentrations in the deepest layers of a normal size human kidney within a reasonable time (10 hours)

The aim of this study was therefore to determine the suitability of kidney preservation at 5 C using oxygen pressures of 50 25 and 15 ATA

## MATERIAL AND METHODS

Twelve female pigs of the Danish Landrace breed aged 4 to 5 months and weighing 42 to 76 kg at the time of surgery were used for the study The average kidney weight was 160 g (1 6–175 g) The animals were fed during the experimental period with a standard fodder mixture (3) They were weighed once a week and the daily administration of fodder was calculated on the basis of the body weight Unlimited quantities of water were permitted

*Preservation of the kidney*

All autotransplanted kidneys were stored for 24 hours at 5 C and at hyperbaric oxygen pressure The handling of the kidney from the time of excision to recirculation is summarized in Table I As soon as possible after excision

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Table I *Summary of the treatment of twelve kidneys during the period from removal to recirculation*
All the kidneys were kept under hypothermia (5 C) and hyperbaric oxygen for 24 hours

| Pig no | Warm ischaemia (min) | Time for perfusion (min) | Temperature at the end of perfusion (C) | Tis U Sol (ml) | Oxygen pressure (ATA) | Time for increasing pressure (min) | Time for decreasing pressure (min) | Time for suturing renal vessels (min) | Temperature just before recirculation (C) |
|---|---|---|---|---|---|---|---|---|---|
| 40 | 11 | 12 | — | 500 | 15 | 25 | 20 | 33 | 33 |
| 42 | 3 | 7 | — | 500 | 15 | 30 | 75 | 42 | — |
| 44 | 5 | 6 | — | 500 | 15 | 35 | 35 | 40 | — |
| 45 | 10 | 6 | 9 | 500 | 15 | 30 | 15 | 30 | 27 |
| 46 | 7 | 33 | 7 | 500 | 15 | 30 | 25 | 37 | 28 |
| 47 | 7 | 30 | 9 | 500 | 15 | 30 | 25 | 38 | 32 |
| 49 | 6 | 5 | — | 500 | 15 | 30 | 20 | 38 | 30 |
| 50 | 14 | 7 | 7 | 500 | 15 | 30 | 20 | 36 | 26 |
| 51 | 10 | 6 | — | 500 | 50 | 5 | 720 | 42 | — |
| 52 | 8 | 21 | — | 500 | 50 | 5 | 1410 | 32 | — |
| 53 | 6 | 5 | — | 500 | 25 | 30 | 80 | 50 | — |
| 54 | 5 | 5 | — | 500 | 25 | 60 | 110 | 28 | — |

the kidney was perfused in a cold room (5 C) with 500 ml TIS-U SOL (containing NaCl 3 5 g, KCl 0 4 g, Na HPO 2H₂O 0 058 g, KH PO 0 0675 g, glucosum NFN 1 0 g, dextranum hydrolysatum NFN 50 g and aqua sterilisata ad 1000 g) with addition of 2500 I U heparinum NFN (Heparin Leo) 25 mg papaverini sulfas NFN and 540 mg tris-(hydroxy methyl) amino-methane The pH of this solution was adjusted to 7 3 by means of hydrochloric acid. The perfusion pressure was about 130 cm water The on fluid had been oxygenated for at least 30 and had an oxygen tension varying between 550 and 650 mm Hg (Beckman oxygen electrode) The temperature 1 cm inside the kidney was measured during perfusion in four cases with a thermistor (ISC E.L. Laboratories Copenhagen) During storage in the pressure chamber the kidney was placed on a rack which permitted contact between the oxygen and the whole surface of the kidney (7) The times for pressure increase and decompression are shown in Table I After removal from the pressure chamber the kidney was reimplanted, as described below During this process the kidney was not cooled externally In six cases the temperature 1 cm inside the kidney was measured with a thermistor just before recirculation (Table I)

The experimental animals were divided into three groups

*Group 1* The excised kidneys were stored at 50 or 25 ATA oxygen pressure and contralateral nephrectomy was performed in conjunction with transplantation (4 pigs)

*Group* The excised kidneys were stored at 15 ATA oxygen pressure and contralateral nephrectomy was performed in conjunction with transplantation (? pigs)

*Group 3* The excised kidneys were stored at 15 ATA oxygen pressure and contralateral nephrectomy was performed three weeks after transplantation (6 pigs)

*Surgical technique*

Renal autotransplantation with uretero-vesical anastomosis was performed followed by contralateral nephrectomy either immediately after the transplantation (groups 1 and 2) or three weeks later (group 3) Details of the surgical technique have been published previously (4) Despite the fact that development of ureteral stenosis was observed following uretero vesical anastomosis in pigs (4) this technique was used in the present study because oedema in the preserved ureter proved to be a serious technical obstacle to the application of uretero-ureteral anastomosis At the time of contralateral nephrectomy in the group 3 pigs the autotransplanted kidney was inspected and a biopsy was taken

*Post-operative studies*

*Blood analyses* During the first week after surgery blood samples were taken every other day and subsequently once a week for the following four months. The pH and haematocrit values were determined, together with the concentrations in plasma of creatinine urea, sodium and potassium and the activity of lactic acid dehydrogenase

*Kidney function* The clearances of inulin endogenous creatinine urea, para aminohippuric acid (PAH) and the excretion percentages of water sodium potassium and chloride were determined The experiments were performed on unanaesthetized animals on the first occasion ten days after contralateral nephrectomy and subsequently every third week Each experiment consisted of at least three periods of 0 min and the last experiment concluded with three periods of 20 min to determine the Tm for PAH Three days after the last clearance experiments the extraction percentage for PAH was determined after which the animals were killed Details of the doses of test substances, the technique and the calculation methods used have been published previously (3)

*Analytical methods* The lactic acid dehydrogenase activity (LDH) was assessed by the method of Laursen (6) (fluorimetric determination at 37 C of nicotinamide adenine-d nucleotide (NAD) the unit used being mol h ml) The other methods have been described previously (4)

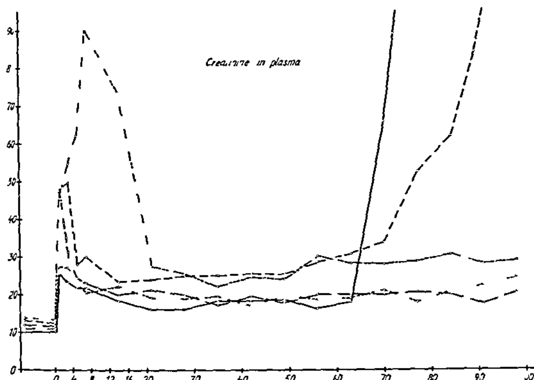



*Fig 1* Concentration of creatinine in plasma 0–98 days after 2nd nephrectomy Ordinate creatinine in plasma µg–ml Abscissa days after 2nd nephrectomy O– –O pig no 44 O– – – – –O pig no 45 O –O pig no 46 O— — —O pig no 47 O—O pig no 49

*Postmortem examinations* The animals were killed and bled, and postmortem examination was performed. The kidneys were weighed. Kidney tissue was removed for histological examination and fixed in neutral buffered formalin, as were also the biopsies taken during contra lateral nephrectomy (group 3) Paraffin wax sections were stained with iron hematoxylin–v Gieson, and the periodic acid Schiff reaction was carried out according to McManus and Mowry (11)

## RESULTS

### Group I

*Initial behaviour*

After removal from the pressure chamber the kidneys, which were stored at 50 ATA pressure were distended and revealed palpable crepitation due to liberation of the physically bound oxygen during decompression in the pressure chamber This was also the case when decompression took place gradually over a period of 24 hours (pig no 52) Kidneys stored at 25 ATA oxygen pressure had the same macroscopic appearance and consistency before and after application to the pressure chamber After recirculation the kidneys stored at 50 ATA rapidly became cyanotic and strongly oedematous whereas those stored at 25 ATA gradually became pink and grew oedematous after 10–15 min In all cases urine production started a few minutes after recirculation and continued throughout the surgery The concentrations of potassium and sodium in the urine collected during the surgery corresponded to the plasma concentrations

*Subsequent function*

Production of urine ceased a few hours after the surgery and after three to five days the animals died in uraemia The LDH concentration in plasma was measured in three of the pigs. In pigs nos 51 and 52 (50 ATA) the concentrations were between 162 and 180 units, and in pig no 53 (25 ATA) the concentration increased to 120 units The average concentration in plasma in a normal material of 12 pigs was 44 ± 6 s d

### Postmortem examinations

*Macroscopical findings*

The surface of the kidneys stored at 50 ATA oxygen pressure was dark and mottled and on the cut surface it was seen that the outer 6–7 mm

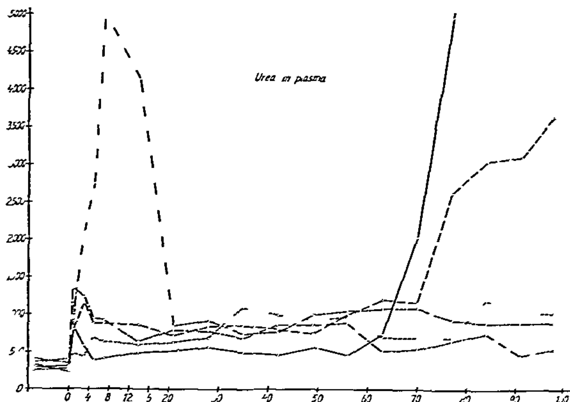



*Fig 2* Concentration of urea in plasma 0–98 days after 2nd nephrectomy Ordinate: urea in plasma μg ml. Abscissa. days after 2nd nephrectomy ○--○ pig no 44 ○------○ pig no 45 ○--○ pig no 46 ○----○ pig no 47 ○—○ pig no 49

of the cortex was totally necrotic and separated from the deeper parts of the cortex by a haemorrhagic zone There were dry yellowish striped areas in the section between cortex and medulla The preserved ureters were extremely oedematous and in pig no 52 the ureter was partially necrotic The arterial and venous anastomoses healed without any sign of stenosis or thrombus formation

The kidneys which were stored at 25 ATA oxygen pressure were slightly enlarged The surface was greyish-brown with dark brown patches and small yellowish areas The latter extended through most of the cortex and could be seen on the cut surface in the form of radial yellowish stripes There was free passage from the pelvis to the bladder and the arterial and venous anastomoses healed without any sign of stenosis or thrombus formation

Table II *Average renal clearances in five pigs 10–94 days after 2nd nephrectomy*

The bracketed figures indicate minimal and maximal values of single experiments

| Days after 2nd nephrectomy | Body weight (kg) | Haematocrit ( ) | Diuresis (ml min) | Inulin (ml min 10 kg b wt.) | Endogen creatinine (ml min 10 kg b wt.) | Urea (ml min 10 kg b wt.) | PAH (ml min 10 kg b wt.) |
|---|---|---|---|---|---|---|---|
| 10 | 63 (5.–71) | 34 (31–38) | 2.8 (1.–5 2) | 8 (2–12) | 10 (.–15) | 5 (1–8) | 33 (5–.6) |
| 31 | 82 (67 93) | 33 (.8–39) | 5 4 (1 7–11 3) | 10 (7–14) | 12 (9–15) | 7 (5–10) | 35 (.6–4 ) |
| 52 | 92 (75–103) | 35 (31–44) | 2.1 (1 1–6 1) | 9 (6–14) | 11 (6–15) | 5 (.–9) | 35 (20–5.) |
| 73 | 105 (87–1.1) | 35 (33–38) | 3 7 (3 4–4 1) | 6 (2–11) | 9 (3–13) | 4 (1–7) | 6 (6–53) |
| 94[a] | 114 (100–125) | 31 (.4–34) | 4 0 (3 4–4 5) | 7 (1–12) | 9 (2–14) | 4 (1–7) | 37 (5–75) |

[a] Pig no .9 not included.

*Microscopical findings*

All the kidneys showed severe degenerative tubular changes The cortex of the kidneys stored at 50 ATA was almost totally necrotic.

### *Group 2*

*Initial behaviour*

The appearance and consistency of the kidneys was the same before and after insertion in the pressure chamber Immediately after establishment of the blood circulation the kidneys became pink in colour and urine production started within a few minutes After 10–15 min oedema developed in the kidney The pink colour and urine flow were maintained during surgery

*Subsequent function*

The production of urine ceased a few hours after the operation and the animals died after three to five days in uraemia

*Postmortem examinations*

*Macroscopical findings*

The kidneys were slightly enlarged The surface was greyish brown The cut surface was pale with greyish yellow radial stripes in the cortex The pelvis was slightly dilated but there was free passage through the ureter to the bladder Arterial and venous anastomoses healed without any sign of stenosis or thrombus formation

*Microscopical findings*

There was severe tubular degeneration

### *Group 3*

*Initial behaviour*

The appearance of the kidneys after removal from the pressure chamber and after re-establishment of the circulation was as found in the group 2 animals There was copious flow of urine The concentrations of potassium and sodium in the urine corresponded to the concentrations in plasma

| Effective renal plasma flow (ml min/10 kg b wt ) | Effective renal blood flow (ml min/10 kg b wt ) | Clearance ratios | | |
|---|---|---|---|---|
| | | Cr/In | Urea In | In/PAH |
| 36 (5–61) | 55 (7 88) | 1 2 | 0 6 | 0 _4 |
| 38 ( 8–46) | 57 (42–74) | 1 2 | 0 7 | 0 28 |
| 38 (22 54) | 58 (39–79) | 1 2 | 0 5 | 0 _6 |
| 8 (7 58) | 43 (11–82) | 1 5 | 0 7 | 0 23 |
| 40 (5–82) | 58 (7–120) | 1 3 | 0 6 | 0 19 |

*Subsequent function*

On inspection of the transplanted kidneys three weeks after transplantation all of them (except the kidney from pig no 50) had normal size form, colour and consistency In pig no 50 there was an infarct involving about half of the kidney On account of this complication the findings in that animal are not included in the results of the renal function examinations

Microscopy of the biopsies from the kidneys three weeks after transplantation revealed varying degrees of interstitial fibrosis In some instances there was hydropic change and hyalinedroplet formation in the proximal tubes The kidney from pig no 45 showed dilatation of the capsular spaces tubules and collecting ducts

*Concentrations of creatinine urea LDH sodium and potassium in plasma*

Figs 1 and 2 show the concentrations of cre ti nine and urea in plasma (μg ml) respectively

There was a decreasing tendency in the sodium concentration from the beginning to the end of the experimental period The average value during the first eight days after transplantation was 141 ±4 S D During the last month of the experimental period it was 134 ± 4 S D The potassium concentration was constant at 5 0 ± 0 8 S D throughout the period The pH was constant at an average of 7 31 ± 0 07 S D The activity of LDH in plasma was determined at the first nephrectomy and during the following four weeks In this period there was no significant increase in relation to the values on the day of nephrectomy or to the values in the normal material mentioned previously

*Clearances of inulin endogenous creatinine urea and PAH*

Table II gives the average values for the clearances of inulin endogenous creatinine urea and PAH for the effective renal plasma flow (RPF$_{eff}$) and total renal blood flow (RBF$_{eff}$) The clearance ratios are also stated It will be seen that the values for inulin endogenous creatinine and urea clearance were highest

Table III *Renal clearances and total renal blood flow in four pigs 94 days after 2nd nephrectomy*

| Pig no | Clearance: Inulin ml/min/10 kg b wt | Inulin ml/min/100 g kidney | Endogenous creatinine ml/min/10 kg b wt | Endogenous creatinine ml/min/100 g kidney | Urea ml/min/10 kg b wt | Urea ml/min/100 g kidney | PAH ml/min/10 kg b wt | PAH ml/min/100 g kidney | Extraction ( ) | Total renal blood flow ml/min/10 kg b wt | Total renal blood flow ml min/100 g kidney |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 44 | 1 | 3 | 2 | 7 | 1 | 3 | 5 | 17 | 17 | 39 | 140 |
| 45 | 6 | 20 | 9 | 30 | 4 | 13 | 29 | 103 | 73 | 65 | 233 |
| 46 | 8 | 31 | 10 | 38 | 4 | 17 | 37 | 147 | 62 | 95 | 379 |
| 47 | 12 | 35 | 14 | 40 | 7 | 21 | 75 | 200 | 62 | 193 | 515 |

on the 31st day after the second nephrectomy The lower values on the 73rd and 94th day were due to particularly low values in pigs nos 49 and 44 Pig no 49 died in a severely uraemic condition before the last clearance examination and therefore the results on the 94th day refer to four pigs only Since the animals were killed immediately after the last clearance experiment it was possible to calculate the clearances both per 10 kg body weight and per 100 g kidney tissue The results are given in Table III which also shows the extraction percentage for PAH used for calculating the total renal blood flow ($RBF_{total}$)

*Maximal tubular excretion of PAH*

In the last experiment the Tm of PAH was determined and the results for each individual pig are shown in Table IV both per 10 kg body weight and per 100 g kidney tissue The table also includes the plasma concentrations of PAH at which the Tm determinations were performed

*Excretion of water sodium potassium and chloride*

The average excretion percentages for water sodium potassium and chloride are shown in Table V The average values for the whole period for sodium potassium and chloride were 1 93 64 and 4 72 respectively

*Postmortem examinations*

*Macroscopical findings*

The kidneys from pigs nos 44 and 45 were greyish brown in colour very firm and difficult to decap ulate Both kidneys were strongly hydronephrotic with complet ly flattened papillae and marked reduction of the kidney tissue particularly of the medulla In both pigs there was a considerable narrowing of the opening of the ureter into the bladder Here th• diameter of the ureter was about 1 mm The kidneys from these two pigs weighed 281 and 371 g and the relative kidney weights were 0 28 and 0 29 % Pigs nos 46 and 47 had greyish brown kidneys which could be decapsulated readily The kidney from pig no 46 was slightly hydronephrotic with flattened papillae and slightly firm consistency The kidney from pig no 47 had normal papillae and normal consistency In both these pigs the ureter was moderately narrowed at the entry into the bladder The lumen at that site was about 3 mm The

Table IV *Clearance and maximal tubular excretion (Tm) of para aminohippuric acid 94 days after 2nd nephrectomy*

| Pig no | Body weight (kg) | Kidney weight (g) | Relative kidney weight ( ) | Plasma concentration of PAH (μg/ml) | PAH clearance (ml/min/10 kg b wt ) | Inulin clearance (ml/min/10 kg b wt ) | Tm (mg/min/10 kg b wt ) | Tm (mg m n/100 g kidney) |
|---|---|---|---|---|---|---|---|---|
| 44 | 101 | 281 | 0 28 | 1460 | 4 | 1 | 3 | 11 |
| 45 | 111 | 317 | 0 29 | 1620 | 12 | 6 | 11 | 37 |
| 46 | 120 | 300 | 0 25 | 1140 | 14 | 8 | 8 | 33 |
| 47 | 125 | 468 | 0 37 | 1270 | 23 | 12 | 17 | 45 |

Table V *Average renal excretion of electrolytes in five pigs 10 to 94 days after 2nd nephrectomy*

The bracketed figures indicate min and max values for single experiments

| Days after 2nd nephrectomy | Body weight (kg) | Diuresis (ml, min) | Inulin clearance (ml min/10 kg b wt) | Excretion Water | Sodium | Potassium | Chloride |
|---|---|---|---|---|---|---|---|
| 10 | 63 | 2.8 | 8 (2 12) | 11 6 | 3 8 | 76 | 6 1 |
| 31 | 82 | 5 4 | 10 (7–14) | 7 5 | 0 7 | 51 | 2 4 |
| 52 | 92 | 2 1 | 9 (6–14) | 5 1 | 0 7 | 43 | 1 8 |
| 73 | 105 | 3 7 | 6 (2–11) | 7 6 | 2 2 | 68 | 4 0 |
| 94 | 114 | 4 0 | 7 (1–12) | 13 3 | 2.8 | 86 | 5 |

P g no 49 not included

kidney from pig no 46 weighed 300 g and the relative kidney weight was 0 25 % the corresponding values for pig no 47 were 468 g and 0 37 The kidney from pig no 49 was greyish yellow very firm in consistency and impossible to decapsulate The pelvis was very distended and the papillae totally flattened The ureter was cord shaped throughout the whole length with a lumen of about 0 5 mm The kidney weighed 529 g and the relative kidney weight was 0 50 %

*Microscopical findings*

In the kidneys of pigs nos 44 45 and 49 there was *pronounced interstitial fibrosis and dilatation* of the tubules and capsular spaces The kidneys of pigs nos 46 and 47 showed slight interstitial fibrosis

## DISCUSSION

The results from groups 1 and 2 in the present study where contralateral nephrectomy was performed simultaneously with the transplantation show that such severe damage was caused to the kidneys under preservation with 15 25 and 50 ATA that the pigs could not survive Oxygen pressures of 50 and 25 ATA (group 1) caused more pronounced damage to the tissue than 15 ATA This finding is presumably related to the very high concentration of oxygen in the superficial layer of the kidney and to the mechanical bursting of the tissue by liberation of the physically bound oxygen during decompression (7) These observations are in accordance with the results of Ladaga et al (5) which were published after our experiments were *performed In experiments with* oxygen pressures of 2 5 10 20 and 30 ATA Ladaga et al found the least pronounced histological changes and the highest renal clearances following delayed contralateral nephrectomy when using 5 and 10 ATA On the basis of the results from groups 1 and 2 in the present study renal clearance experiments with postponed contralateral nephrectomy were carried out only on pigs whose kidneys had been stored at 15 ATA (group 3)

In all the group 3 pigs the content of creatinine and urea in plasma increased during the first days after contralateral nephrectomy (Figs 1 and 2) Except for pig no 45 the concentrations of creatinine and urea in plasma fell after five to ten days to stable levels which is in agreement with the findings of other authors (1 5 9 13) These concentrations were maintained throughout the whole exp rimental period except in pig nos 44 and 49 in which the increase about ten weeks after contralateral nephrectomy was due to the development of ureteral stenosis During the period from 20–50 days after contralateral nephrectomy the creatinine concentration in plasma was constant at an average of 21 $\mu g/ml \pm 3$ S D while in the pigs with autotransplanted not long term preserved kidneys (4) the concentration was 17 $\mu g/ml \pm 0.25$ S D ($0.01 < p < 0.05$)

The inulin endogenous creatinine urea and PAH clearances determined 52 days after contralateral nephrectomy were 60–80 % of the corresponding clearances in pigs with autotransplanted not long term preserved kidneys (4) The highest clearance values were obtained in pig no 47 which showed no signs of hydronephrosis The results are *in agreement with those of* Ladaga et al (5) who using 10 and 20 ATA oxygen pressure *for* 24-hour preservation of dog kidneys found decreased creatinine and PAH clearances when compared to autotransplanted not long term preserved kidneys

The excretion percentages of sodium potassium and chloride were 6 2 and 4 times higher re spectively than in the control group (4) The loss of sodium from the kidney in the preserved group caused a significant decrease in the sodium con centration in plasma during the observation period whereas the potassium concentration remained constant

There were large variations in the maximal tubular excretions of PAH and the excretion percentages for PAH determined at the end of the experimental period Pig no 44 with pronounced patho-anatomical alterations and pig no 47 with few changes had Tm of 3 and 17 mg/min/10 kg body weight respectively while the Tm for auto-transplanted not long term preserved kidneys (4) was 19 mg/min/10 kg body weight $\pm 5\ 6$ S D The extraction percentages varied from 17 to 73% while the value in normal pigs is 87% (3)

The average filtration fraction was $0\ 26 \pm 0\ 06$ S D which is significantly lower than in auto-transplanted not long term preserved kidneys (4) where it was $0\ 30 \pm 0\ 05$ S D $(0\ 01 < p < 0\ 02)$

The average clearance ratio for endogenous creatinine and inulin was $1\ 35 \pm 0\ 25$ which is gnificantly higher than in autotransplanted not term preserved kidneys where it was $1\ 15 \pm 0\ 17$ $(p < 0\ 01)$ (4) and in normal pigs where it is $0\ 96 \pm 0\ 14$ S D $(p < 0\ 01)$ (3)

On the basis of own experiments and the ex perience of others the following conclusions are apparently relevant

Preservation for 24 hours at 25 and 50 ATA oxygen pressure which theoretically should permit a reasonably brief ischaemic period causes such extensive damage to the tissue that the method cannot be used in the clinic

Preservation for 24 hours at 15 ATA oxyg n pressure causes a transient acute reduction in renal function which in the clinic would presumably call for dialysis treatment The function of the kidney apparently remains permanently reduced Furthermore on account of oedema in the pre served ureter it is difficult to perform a uretero-ureteral anastomosis

## ACKNOWLEDGEMENTS

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## REFERENCES

1 *Ackermann, J R W* & Barnard, C. N Successful storage of kidneys Brit J Surg. 53 525 1966
2 Basso A *Calne* R. Y & Hopkinson, W L Renal preservation with hyperbaric oxygenation and hypothermia Brit. J Urol. 39 276 1967
3 Gyrd Hansen N Renal clearances in pigs Acta vet. scand 9 183 1968
4 Gyrd Hansen, N Lund F Iversen Hansen, R., Fer nandes, A Kemp E. Løkkegaard H & Rasmussen, F Renal clearances in unilaterally nephrectomized pigs and in pigs with an autotransplanted kidney Acta med scand 186 383 1969
5 Ladaga L. G Cault, M H Sheldon, H & Maclean, L. D Preservation of the dog kidney J surg. Res. 8 495 1968
6 Laursen, T A fluorimetric method for measuring the activity in serum of the enzyme lactic dehydrogenase Scand J clin Lab Invest. 11 134 1959
7 *Løkkegaard, H* Kidney preservation with hypothermia and hyperbaric oxygen. I The diffusion of oxygen into the kidney Acta med scand 187 189 1970
8 Makin, G S & Howard J M Hyperbaric oxygena tion and profound hypothermia to preserve canine kidneys Arch Surg 91 568 1965
9 Manax, W G Bloch J H *Longerbeam* J K. & Lillehei R. C Successful 24 hour in vitro preserva tion of canine kidneys by the combined use of hyper baric oxygenation and hypothermia Surgery 56 75 1964
10 Manax, W G Largiader F & Lillehei R. C Whole canine organ pres rvation J Amer med Ass 196 1121 1966
11 McManus J F A & Mowry R. W Staining meth ods Histologic and histochemical p 1 6 Hoeber New York 1960
12 Mirkovitch V Pernard M Hadra, S & Hegi F Conservation du rein in vitro sous hypoth rmie et oxygene hyperbare Schweiz med Wschr 96 1301 1966
13 Ringdal R Linder E & Pfenninger E Extra *corporeal preservation of canine* kidney with sub-sequent reimplantation and contralateral n phrectomy Acta chir scand 33 5 1967

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# THE INFLUENCE OF INFECTION ON THE DEGREE OF BONE MARROW INSUFFICIENCY

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*Ab t ct* The clinical significance of infection in bone marrow failure has been demonstrated in the case histories of five patients with panmyelopathy In these patients the development of infections was associated with a hema tolog c relapse With suc‑ssful treatment of infections a marked improvement of the hematologic status occurred, characterized by at least a decreas in transfusion require ment and sometimes an increase in leucocyte count and the number of platelets. In one case (no 1) a complete remission occurred. Apparently infection may lead to acute bone marrow failure in patients with panmye lopathy possibly by increasing the load on a damaged organ which is already working at the maximum of its limited capacity Adequate treatment and pre ention of infections is therefore of major importanc for th hemato ogic status of those patients.

Panmyelopathy or aplastic anemia which is char acterized by impaired production of blood cells is more or less independent of the degree of cel lularity of the bone marrow and may be due to different causes This disorder—while often dan gerous—is not always associated with clinical symptoms If one borrows the terminology used for describing the functional status of other or gans for example the heart one can sp ak of diminished bone marrow reserve or latent bone marrow failure in patients with asymptomatic panmyelopathy With more severe bone marrow damage manifest insufficiency may develop Then the clinical syndrome of bone marrow failure is found. This is characterized by the development of signs and symptoms of anemia the occurrence of external and internal hemorrhage and the oc currence of infections

Decreased resistance as indicated by a high in cidence and often rapid progression of infections is generally accepted as a major consequence of bone marrow disease The present paper deals with anoth r aspect of infection in panmyelopathy namely the effect of infection on the degree of bone marrow insufficiency The clinical signifi cance of this phenomenon will be demonstrated by some case histories Thes were selected from the histories of patients which have recently been reviewed in a thesis prepared by one of us (13)

## MATERIAL AND METHODS

The diagnosis of panmyelopathy was based o cli al examination and on s udies of the peripheral b ‑ an the bone marrow and was made in patien s with p cytopenia whi h could not be ascr bed to n reased d struction of peripheral blood cells or to disorder ‑ iated w th splenomegaly Primary or secondary m lign t disease in olving the bone marrow was also ex luo d

The bone marrow wa studied both in sme r obtain d by sternal puncture and n section obtained by n ed e biopsy (7) Skeletal X rays stud es we e performed

Peripheral blood cell were counted by means of a Coulter counter in the case of red cells and wh t cells while platelet counts were performed in triplicate in venous blood collected in EDTA containin siliconiz d syringes by the method of Feissly and Lud n (5)

The survi al of periphe al blood cells wa n estig ted by means of radioactive chrom um tagging of erythro‑cytes and thrombocytes The method of Aster and Jandl (1) was used for determ n ng the platelet lif span and the modification described by V eger et al (1 ) for estimat ng red cell surv val

For the detection of red cell wh te cell and platelet antibodies, direct Coombs tests as well as direct and indirect ant globulin consumption tests were performed, always with negati e results.

In addition liver and kidney funct on studies were performed.

In e ery patient we tried to determine the etiology of the bone marrow damage by taking a careful h story

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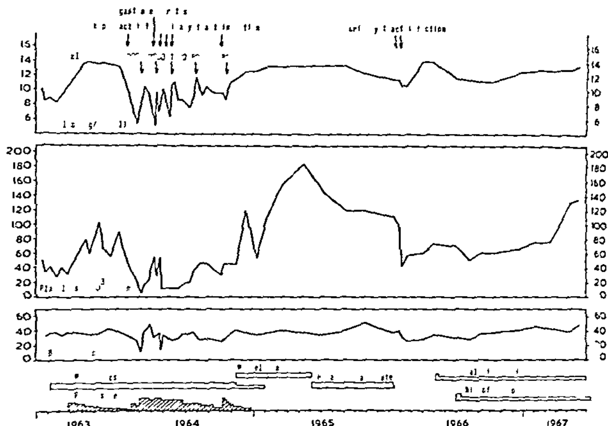



*Fig 1* In this young woman with bone marrow hypoplasia manifest infections were accompanied by a relapse of the pancytopenia. After treatment a gradual improvement of the blood values was observed (case 1)

Inquiries were made about drug intake and about exposure to potential harmful agents in the professional and home environment.

## CASE REPORTS

*Case 1*

This young woman was born in 1938 and was admitted in 1963 to our department after an initial observation elsewhere. Pancytopenia on the basis of bone marrow hypoplasia was found. The platelet life span was normal.

In the preceding year she had taken part in repairing her parental home and had painted the entire interior. It was not possible to establish the nature of the solvents and thinners used. She also had taken analgesic preparations containing acetylsalicylic acid and phenacetin at irregular intervals for several years.

The patient was treated with prednisone (25 mg per day) and methandrostenolone (Dianabol®) (10 mg per day), while an oral contraceptive (Lyndiol®) was administrated to control menorrhagia. In the first six months the blood picture improved gradually. Then the patient developed infections of the respiratory, gastrointestinal and urinary tract. Multiple blood transfusions (18 units over 8 months) were required to maintain an adequate hemoglobin level during this period.

When relapse of the urinary tract infection due to Proteus mirabilis occurred, continuous treatment with mandelic acid and later on with hexamine mandelate was given, while prednisone and methandrostenolone were stopped. Subsequently a gradual improvement of the blood values occurred and the peripheral blood count became completely normal (Fig. 1).

After a period of one year on this regime the urinary tract infection with Proteus mirabilis recurred once again. This episode of acute inflammation was again accompanied by a relapse of the pancytopenia. The hemoglobin level decreased from 13.6 g per 100 ml before this episode to 11.6, the leucocyte count from 5000 to 3000 per mm³ and the platelet count from 1_0 000 to _0 000 per mm³. After treatment of this acute episode of pyelonephritis we continued with a maintenance regime of nalidixic acid (Negram®) 2 g daily and nitrofurantoin (Furadantine®) 100 mg daily which has been given since June July 1966. Since then the urine has remained sterile and the peripheral blood picture gradually improved once again. The last values counted in March 1968 were: hemoglobin level 13.0 g per 100 ml, white blood cells 6700 per mm³ and platelets _40,000 per mm³.

*Case*

*This woman, born in 1894 had since her youth suffered from allergic diseases (vasomotor rhinitis and bronchial*

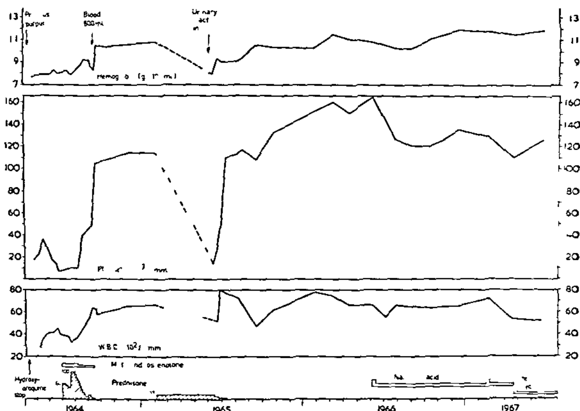



*Fig.* After treatment of a serious urinary tract infection the hemoglobin level and platelet count increased markedly in case …

asthma). In 1964 she developed itching and purpura of the legs while she was being treated with hydroxychloroquine (Plaquenil ®) because of rheumatoid arthritis. She had received a total amount of 4 g of this drug. She was admitted three weeks afterwards; at that time pancytopenia was detected. Bone marrow sections showed a predominantly hypoplastic picture with many fat cells and only a few cellular areas. The survival of red cells and platelets was found to be normal.

She was treated with prednisone and methandrostenolone. Four months after admission a gradual increase of the leucocyte and the platelet count was observed. She maintained an adequate hemoglobin level after the initial administration of two units of blood. It should be pointed out that this remission occurred after the dose of prednisone had been decreased.

Before the remission was complete she developed a serious urinary tract infection with high fever caused by paracolon bacteria. The intravenous pyelogram showed marked hydronephrosis and dilatation of the ureter to the uretero-vesical junction on the right side. Many years earlier the left kidney had been removed, probably because of renal calculi. Kidney function studies showed essentially normal values (urea 55 mg/100 ml, creatinin 0.86 mg/100 ml, creatinine clearance 78 ml/min).

After treatment of this infection with tetracycline and nitrofurantoin the platelet count increased markedly. Initially the hemoglobin level also showed a rapid rise, but a hemolytic episode probably due to an iodine containing contrast medium prevented a further increase during some weeks. The patient was given maintenance treatment with mandelic acid (1. g daily), methionin (8 g daily) and later on with hexamine mandelate (4 g daily) in order to keep the urine sterile. Subsequently we changed to maintenance treatment with nalidixic acid (Negram ®) 2 g daily. On this antibacterial regime she had during nine months no signs or symptoms of an urinary tract infection, and cultures of the urine were also sterile. Later on asymptomatic bacteriuria with paracolon bacteria sensitive to tetracyclin occurred and therefore we recently started with 1 g of tetracycline daily. Thereupon the urine became sterile. During this period she has maintained a nearly normal peripheral blood picture (Fig. 2).

## *Case 3*

This man, born in 1897, was admitted in 1959 because he had for some time been increasingly tired and dyspnoic. He was found to suffer from pancytopenia with a hypoplastic bone marrow. In addition increased hemolysis appeared to exist, expressing itself as hyperbilirubinemia

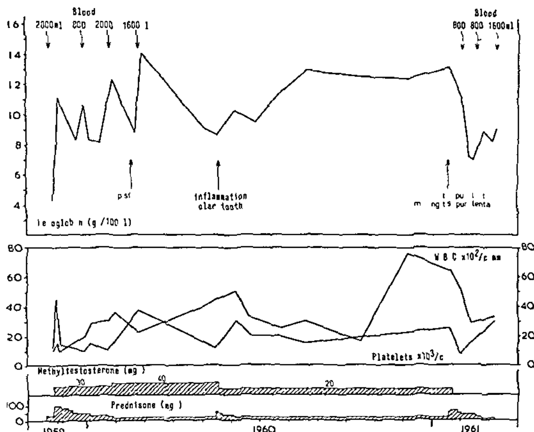



*Fig 3* The effect on the blood values of an abscessed tooth sinusitis and subsequent meningitis in a man with pancytopenia (case 3)

and urobilinuria The reticulocyte count however was low (0 5–0 7 )

He was treated with prednisone and methyltestosterone Because of heavy blood loss due to epistaxis he was repeatedly transfused with fresh blood collected in siliconized bottles Unfortunately he developed steroid diabetes for which insulin administration was required

A second admission was necessary fourteen weeks later because of a high fever which was caused by inflammation of a carious molar associated with extensive collateral edema This process subsided after the administration of tetracycline 2 g daily during 18 days Subsequently the hemoglobin level and the white blood cell count increased gradually to normal values, but the number of platelets remained low (Fig 3)

Admission was once again necessary because of attacks of severe headache and jaundice The jaundice was considered to be the result of intrahepatic cholestasis caused by the androgen. The headache which was accompanied by fever was found to be due to a purulent pan sinusitis The inflammation did not subside in spite of treatment with several antibiotics (tetracycline chloramphenicol penicillin and streptomycin) Even though repeated transfusions were given the hemoglobin level decreased in the course of six weeks from 13 g per 100 ml to 7 g per 100 ml Shortly before the patient died he complained of severe headache and the temperature rose sharply An autopsy showed the existence of extensive purulent meningitis which had extended from the ethmoidal and sphenoidal sinusitis and was considered to be the cause of death In addition there were disseminated foci of bronchopneumonia and bilateral pyelonephritis as well as Aspergillus fumigatus containing abscesses of the thyroid and the kidneys

*Case 4*

This well preserved old man born in 1888 was admitted in 1963 because of complaints of loss of weight, pain in the left upper abdomen and subcutaneous hematomas for the past year Pancytopenia was found associated with enlargement of the liver and the spleen. The red blood cells were normochromic and normocytic and showed anisocytosis and occasionally a tear-drop shape Some normoblasts myelocytes and metamyelocytes were seen The survival of both erythrocytes and thrombocytes was shortened T / for the red cells was 14 days while tagged platelets had all but disappeared from the circulation after 48 hours. At repeated punctures no bone marrow particles were obtained, but a bone marrow section from the iliac crest showed a diffuse increase of connective tissue with isolated foci of hemopoiesis The diagnosis

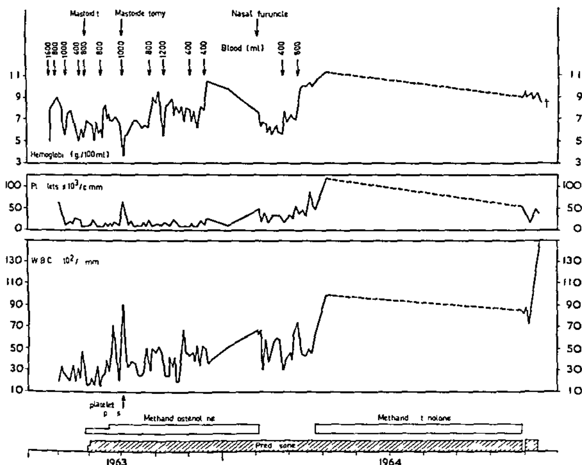



*Fig 4* In case 4 the transfusion requirement decreased after mastoidectomy and once more after treatment of a nasal furuncle

was considered to be bone marrow failure due to myelofibrosis with myeloid metaplasia associated with a moderately increased destruction of peripheral blood cells. Kidney function studies showed essentially normal values (urea 55 mgr/100 ml, creatinine 0 54 mg/100 ml)

The patient was treated with blood transfusions, prednisone (30 mg per day) and methandrostenolone (15 mg per day) Steroid diabetes developed which made treatment with insulin necessary Then we detected mastoiditis which improved insufficiently on administration of antibiotics (rolitetracycline ampicillin) It was therefore necessary to treat the focus of infection in the mastoid by operation. This was done after transfusion with platelet concentrates. Subsequently the transfusion requirement decreased. In the period of 10 weeks preceding the mastoidectomy a total of 16 units of blood was transfused while in the 10 weeks after this procedure only 7 units were needed (Fig. 4) After two months there was a hematologic relapse which was associated with a nasal furuncle from which Staphylococcus aureus was cultured. This infection responded favorably to treatment with tetracycline and once again the blood status improved, even though methandrostenolone was temporarily interrupted for about two months because of an increased bromsulphthalein retention

Later on the patient developed signs of intrahepatic cholestasis which was considered to be due to the anabolic steroid Eventually admission was necessary once again because of severe jaundice The general condition deteriorated quickly and he died with signs of hepatic insufficiency and uremia At autopsy fibrosis of the marrow and enlargement of the spleen which contained hemopoietic foci and infarcted areas, were found There was also cholestasis in the liver pulmonary emphysema and enlargement of the heart with muscular hypertrophy fibrosis and dilatation The kidneys showed only moderate arterio- and arteriolosclerosis These findings did not explain the uremia

*Case 5*

A man born in 1894 was given treatment with phenytoin (total amount approximately 30 g) and phenobarbital (approximately 30 g) because of epilepsy occurring as a

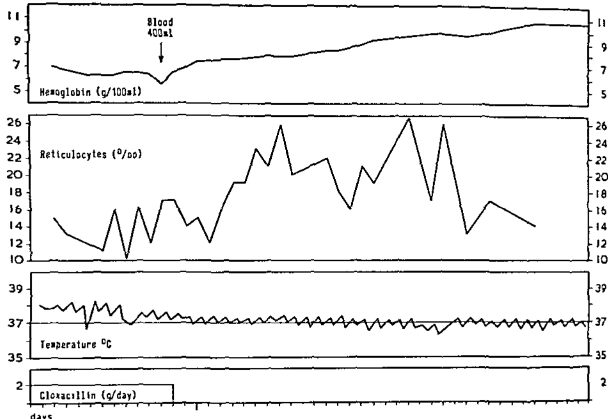



*Fig 5* In case 5 the reticulocyte count increased after successful treatment of a nasal furuncle and after one unit of blood the hemoglobin level continued to increase spontaneously

result of cerebro vascular disease After having used these drugs for six months admission was necessary because of severe anemia associated with leukopenia The anti convulsant drugs were stopped and the patient was given blood transfusions and prednisone as a result of which the hemoglobin concentration increased to more than 10 g per 100 ml At a follow up examination in the out patients department nearly a month later it was more than 11 g per ml Shortly afterwards, however admission was again necessary because of purulent bronchitis and severe pancytopenia (hemoglobin concentration 5 5 per 100 ml leucocyte count 1300 per mm and platelet count 35 000 per mm )

The patient was then admitted to our department. The sternal puncture showed a decreased cellularity Sections from the iliac crest showed some cellular areas in a hypoplastic marrow The red blood cell survival was nearly normal (T ½ = 23 days) After treatment of the infection and administration of blood and prednisone a gradual increase of the hemoglobin concentration occurred.

Nine months later he had a relapse of the anemia he had a nasal furuncle caused by Staphylococcus aureus resistant to penicillin which was successfully treated with cloxacillin (Orbenin®) *The reticulocyte count increased* after one unit of blood the hemoglobin level continued to increase spontaneously (Fig. 5) The leucocyte count, however remained *invariably low (approximately 1000* cells per mm³ with 10 to 20% granulocytes) and the number of platelets also (approximately 30 000 per mm³).

Some weeks later he unfortunately developed an infection once again in this case axillary hydro-adenitis, which was also accompanied by a decrease of the hemoglobin concentration This time treatment with antibiotics did not lead to improvement. After two units of blood the hemoglobin level fluctuated between 8 to 9 g per 100 ml and then showed a tendency to decrease Eventually he developed septicemia due to E. coli which led ad exitum

## DISCUSSION

The development of infections in these five patients is not at all unusual indeed it may be said that infection is still the most serious threat for the patient with bone marrow damage To some extent this may be attributed to the introduction of the transfusion of platelet concentrates as a therapeutic measure which has decreased the incidence of intracranial hemorrhage which was often responsible for the fatal course of panmyelopathy Infections however remain dangerous in spite of recent improvements in antibacterial treatment which have been accompanied by a

changing bacterial pattern of lethal infections The incid nce of fatal staphylococcal disease has decreased and Gram negative bacterial infections are now predominant in these patients (10)

The case histories reported in the present paper have been selected because they d monstrate that the d velopment of an infection in patients with bone marrow damag causes a marked and sometimes rapid decrease of the hemoglobin level In cases 1 and 2 this was accompanied by a decrease of the platelet level Control of the infection may result in an increase of the hemoglobin level and therefore a—sometimes lasting—decrease of the transfusion requirement as well as occasionally an increase of the platelet level

Successful control of the infection sometimes may convert a case of acute bone marrow failure into one of chronic and symptom free panmyelopathy It seems as if one can break a vicious circle in this way

We believe that infection may lead to acute bone marrow failure in patients with panmyelopathy by increasing the load on a damaged organ which is already working at the maximum of its limited capacity A possible explanation of this phenomenon was obtained in animal experiments performed by Bennett et al (2) and Lewis and Trobaugh (6) These workers have observed that isologous marrow cells transplanted into lethally irradiated mice may contain an insufficient population of stem cells when the demand for differentiat d peripheral blood cells is too large

In the patients with a normal bone marrow the effect of an infection on red cell kinetics has been the subject of numerous studies These have d monstrated that the so-called anemia of infection is the result of accelerated red cell destruction associated with an impairment of the erythrocyte production In normal circumstances the bone marrow usually is able to compensate for the degree of red cell destruction associated with infection but when infection is present such a compensatory increase of red cell production does not occur On the basis of the data obtained by Bode et al (3 4) it seems probable that the absence of a compensatory increase of red cell production is due to an effect of the infection on the plasma erythropoietin level In rats in which anemia was induced by removing by heart puncture 50% of the estimated blood volume the usual reaction consisting of an increase of the plasma erythropoietin level does not occur when an inflammatory arthritis is produced by an intraarticular injection of formaldehyde Rats with transfusion induced polycythemia and formaldehyde arthritis were found to be able to respond to injection of exogenous erythropoietin In patients with anemia of infection the administration of cobalt which increases erythropoietin may improve the hemoglobin level It is therefore doubtful whether the impairment of erythropoiesis in the anemia of infection can be considered a manifestation of relative bone marrow insufficiency (9) it is more likely to be due to changes of the humoral control

In aplastic anemia a number of investigators have reported increased levels of erythropoietin both in plasma and the urine As far as we know there are no data about the effect of coexistent infections on the erythropoietin level in patients with this disease In view of the demonstrated inhibition of erythropoietin production by inflammatory processes it seems likely however that the effect of infections on the hemoglobin level may at least to ome extent be caused by a decreased stimulation of red cell production

Another factor which may contribute to the decrease of the hemoglobin level is an increased red cell destruction which is also observed in patients with anemia of infection without preexist nt bone marrow disease In view of the rapid fall of the hemoglobin level observed in some of our patients one has to assume a hemolytic component

The effect of infection in panmyelopathy cannot entirely be explained by assuming a superposition of the so-called anemia of infection on the peripheral blood manifestations of bone marrow damage This is apparent from the sometimes observed clearcut effect on the platelet count At present little is known about the platelet kinetics in infections but it seems likely that the decrease of the platelet level is due to a decrease in production of thrombocytes After an infection it may however take a long time for the platelets to rise to the level existing before the development of the infection This is seen in Fig 4 after the control of the second period of urinary tract infection

Whatever the correct explanation of our clinical experience may be it is clear that adequate treatment of infections can result in a marked improvement of the hematologic status The practical

consequence is that careful tracing of foci of infection such as low grade sinusitis abscessed teeth chronic pyelonephritis etc is of a major *importance in the management of these patients* This should be done at the initial work up of patients with panmyelopathy It should be repeated when there is an increase of the transfusion requirement not explained by blood loss Sometimes surgical exploration of such foci—if necessary under protection of platelet transfusions—is required Adequate treatment of infections with antibiotic or chemotherapeutic drugs can be done only after careful bacteriologic studies including determination of the resistance pattern If the microorganism is sensitive to a drug with bactericidal properties this should be preferred in view of the impaired defence mechanism of the patient. This point was unfortunately sometimes neglected in some of our earlier patients

In the context of this paper preventive measures should also be discussed. Vaccination is not likely to be useful when one is dealing with microorganisms such as Staphylococci and E coli both important in these patients It may be possible *to decrease the incidence of infections* by diminishing the number of bacteria on the skin and in the nasal vestibulum. We therefore advice the use of a bactericidal soap such as povidone (Betadine®) unless there is hypersensitivity to iodine Application of a nasal ointment containing neomycin and gramicidin (Graneodine®) or a similar preparation is also a routine measure since the nasal vestibulum is known to be a choice site for growth of Staphylococcus aureus and since we have repeatedly observed ill-demarcated nasal furuncles in patients with panmyelopathy or leukemia

In patients with chronic bone marrow failure prophylactic oral or parenteral administration of antibacterial drugs is not considered to be advisable in the absence of established infection In this connection we must, however state that it is our practice to keep patients who have had an attack of urinary tract infection for at least a year on a preventive regime by giving urine-acidifying agents (mandelic acid ascorbic acid methionine) or an antibacterial drug or combination of the two

The oral administration of non absorbed antibiotics for the reduction of the intestinal bacterial flora during acute episodes of bone marrow failure induced by cytostatic drugs is at present being evaluated as a preventive measure in certain centers Its potentially great value is not yet established The routine and indiscriminate use of antibacterial drugs to patients with bone marrow failure may however cause definite harm by promoting overgrowth of resistant organisms

*In view of the great significance of the infectious complications of bone marrow failure* we feel that the liberal use of corticosteroids in this condition should be discouraged There are isolated cases in which these preparations are really beneficial but in the majority they are only dangerous (8 11) In the past we have given corticosteroids much more often as can be seen from the reported case histories but now we think it safer to use them only as a last measure and for a short trial period Preferably one should be able to take care of the patient in a specially isolated nursing unit if prednisone is to be used

In discussing the practical consequences of our clinical findings many points have been briefly mentioned, because we believe that the optimal management of patients consists of a complex of *a great many seemingly insignificant details*

## REFERENCES


1 Aster R. H & Jandl, J H. Platelet sequestration in man. I Methods. J clin. Invest. 43 843 1964

2 Bennett, M., Shearer G M., Upton A. C. & Cudkowicz, G Factors affecting the expansion of the stem cell pool in transplantated mouse bone marrow Nature (Lond.) 204 351 1964

3 Bode J J Een klinisch en experimenteel onderzoek over de pathogenese van de anemie bij ontstekingsprocessen in het bijzonder bij rheumatoide arthritis. Thesis University of Groningen, 1961

4 Bode J J., Vegter J J M., Woldring, M G & Nieweg, H. O The pathogenesis of anemia in patients with rheumatoid arthritis. A clinical and experimental study Proceedings of the eighth congress of the European Society of Hematology p 272 S Karger New York 1962.

5 Feissly R. & Ludin, H Action de la cocain sur les plaquettes sanguines. Helv physiol. pharmacol Acta 7 C9 1949

6 Lewis, J P & Trobaugh, F E Haematopoietic stem cells. Nature (Lond.) 204 589 1964

7 McFarland, W & Dameshek, W Biopsy of the bone marrow utilizing the Vim-Silverman needle J Amer med. Ass. 166 1464 19 8

8 Mohler D N & Leavell B S Aplastic anemia an analysis of 50 cases Ann intern Med. 49 3 6 1958.
9 Moore C V The concept of relative bone marrow failure *Amer J Med 23 1 1957*
10 Rapport Gezondheid raad Richtlijnen ter pre entie en bes rijding van ziekenhu sinfecties Staatsuitgeverij The Hague 1967
11 Scott, J L., Cartwright G E. & Wintrobe M M Acquired aplast c anemia an analysis of thirty nine cases and a review of the pertinent literature Medicin (Baltimore) 38 119 1959
1. Veeger W Wo dring, M G van Rood, J J Leeks ma, *C. H W., Verloop M C* & Nieweg, *H O* The value of determination of the site of red cell sequestration in hemolytic anemia as a prediction test for splenectomy Acta med scand 171 507 1962
13 Wijnja, L. Panmyelopathie Een klinisch onderzoek. Thesis University of Groningen 1966

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# SERUM LIPIDS IN AN AMBULATORY DIABETIC CLIENTELE

*Effect of Therapy with Atromidin (Clofibrate)*

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*From Medical Department Central Hospital Uddevalla Sweden*

*Abstract* Serum lipid values—cholesterol and triglycerides—have been determined in 77 male and 107 female ambulatory diabetics mainly middle aged and elderly and most of them in a good or fair diabetic control. Thirty five of 184 patients had cholesterol values exceeding 300 mg% As regards triglycerides 85 had values of more than 150 mg% and 60 more than 200 mg% There was no distinct correlation in this material between cholesterol values and age For triglycerides a certain tendency to positive correlation with age was noticed No correlation could be found between cholesterol and fasting blood sugar A slight tendency in that direction was noticed between triglycerides and fasting blood sugar but of 95 patients with a fasting blood sugar of less than 150 mg% no less than 39 had triglycerides of more than 150 mg% No definite correlation was found between cholesterol and overweight. For triglycerides a distinct such relation to overweight was obvious Forty four hyperlipemic diabetics were treated with clofibrate (Atromidin) with a mean depression of 46 7 mg% for cholesterol and of 80 8 mg% for triglycerides. Hyperlipemic diabetic patients seem to be high risk individuals prone to atherosclerotic complications in which the safe and definitely efficient lipid reducing therapy with Atromidin (clofibrate) is reasonable This lipid reducing therapy might be reserved for not too elderly patients e g. of less than 60 years of age

The existence of a positive correlation between certain serum lipids and atherosclerosis is nowadays scarcely denied by anybody The question whether this relation is causal or not is much more controversial as is well known

Neither would anybody contest that diabetic persons have an increased tendency to develop atherosclerosis If attempts to prevent atherosclerosis are to be made at all there is hardly any group of patients for whom such prophylaxis seems to be more justified than for diabetics

## OWN INVESTIGATIONS

The treatment of diabetes as we perform it at the Medical Department of the Central Hospital of Uddevalla includes besides insulin and peroral diabetic preparations a diet regime that is rather individual According to the degree of overweight the patients are given different degrees of carbohydrate restriction and also restriction of fat intake with a reduction of saturated fats and change to polyunsaturated fats Since several years every patient is provided with written diet instructions about the choice of suitable fats Furthermore we propagandize for physical exercise

One and a half years ago in the autumn of 1967 we started with consistent determinations of serum lipids namely cholesterol and triglycerides Blood samples for that purpose were taken *with the patients fasting, at the same time as the* samples for blood sugar at the patients regular visits to the diabetic office of the Medical Department. In order that the study should not grow beyond our capacity and that of the laboratory only every fourth patient was selected namely those born in the months of January April July and October We have thus collected a material of 184 cases 77 males and 107 females

Cholesterol has been determined according to the method of Liberman and Buchard (11) The normal values are stated by the laboratory to be 150–300 mg For triglycerides the method of Kessler and Lederer (10) has been used with 20–150 mg as normal values As is well known and as stated by Carlson and Lindstedt (4) among others there are significant variations in the normal values of these lipids with for instance not

cholesterol values and age can hardly be said to be obvious with the method for the median used in this material

The distribution of the triglyceride values in different age groups is visualized in Fig 2 Eighty five of 184 patients had values of more than 150 mg% and 64 had more than 200 mg% One male patient 60 years of age had the extreme value of 6570 mg% and a cholesterol value of 960 mg Lipid electrophoresis in this patient revealed an increase of chylomicrons and pre beta lipoprotein and classified the case as hyperlipoproteinemia type V according to Fredrickson et al (7) In this figure as well median values of the different age groups have been plotted using the sex symbols except for the infrequent younger cases The figure shows a trend to higher triglyceride values with higher age

Fig 3 gives the relation between cholesterol and fasting blood sugar The circles represent the mean values for males and females together at

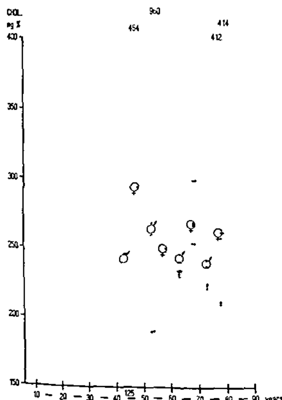



*Fig 1* Cholesterol values in male and female diabetics of different ages The sex symbols represent the mean value of the age group

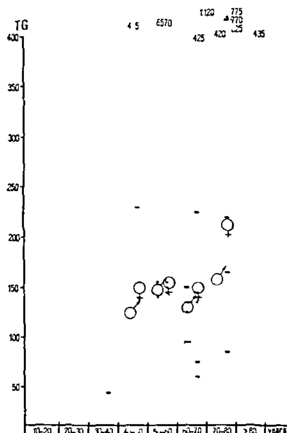



*Fig 2* Triglyceride values in male and female diabetics of different ages The sex symbols represent the mean value of the age group

different levels of fasting blood sugar From this figure it is impossible to find any correlation between cholesterol and fasting blood sugar

As for the triglycerides (Fig 4) the mean values indicate a slight correlation with the fasting blood sugar What appears more significant to us so far is the fact that 39 of 95 patients with a fasting blood sugar of less than 150 mg% had triglyceride values of more than 150 mg%

The correlation between cholesterol and overweight is given in Fig 5 On the abscissa is stated how far body weight in kilograms exceeds body height in cm minus 100 The more to the right the more weight excess The median cholesterol values of the female diabetics are arranged on a somewhat higher level than those of the males but there is hardly any positive correlation between overweight and cholesterol

In a corresponding way the relation between

Table I *Age distribution*

| Age y | No of pats |
|---|---|
| < 20 | 4 |
| 20–29 | 10 |
| 30–39 | 6 |
| 40–49 | 23 |
| 50–59 | 29 |
| 60–69 | 63 |
| 70–79 | 44 |
| > 80 | 5 |
| | 184 |

unimportant differences for age and seasons of the year

In those patients who presented high or suspectedly high values at the first lipid determination (with a value of more than 250 mg% for cholesterol and more than 150 mg% for triglycerides as the borderline) two more determinations of the lipid values have been made subsequently at succeeding diabetic controls A high mean value of the three determinations has then been the reason for therapy with Atromidin (clofibrate) The dose given has been 0 5 g three times daily Broadly speaking Atromidin has been given to patients less than 75 years of age with a cholesterol value of more than 300 mg% and/or a triglyceride value of more than 150 mg% At three later visits to the diabetic office further lipid determinations have been made and the mean value has been calculated

Before the presentation of the results of the therapy with Atromidin some data should be given concerning the 184 primary cases The investigation includes a diabetic clientele fairly advanced in age The pediatric clinic of the hospital takes care of the infantile and most juvenile diabetics These cases are therefore excluded from the present investigation Twenty patients only of the 184 were less than 40 years old (Table I)

## RESULTS

One might reasonably expect the degree of diabetic control to be of importance for the serum lipid values As has been mentioned these have been determined at the routine control visits of the patients to the diabetic office Most patients have had a good or fairly good control In some cases the control of course has been less good One hundred and thirty seven of 183 patients had a glucosuria of less than 20 g per day (Table II)

From Table III it appears which form of therapy the patients had when the first lipid determinations were made As has been mentioned earlier the investigation concerns mainly elderly diabetics and consequently the insulin cases amounted to just a little more than one third of the patients

The following description is an attempt to correlate the serum lipid values with age fasting blood sugar and body weight In Fig 1 the cholesterol values are plotted for different age groups. In every group the male values are found to the left and the female to the right First of all we may draw attention to the fact that 35 of the 184 patients had a cholesterol value of more than 300 mg% The case with 960 mg% had an extremely high triglyceride value as well and will be mentioned later in the discussion of these values For the different age groups the median value has been plotted using the sex symbols for males and females The youngest age groups with a few exceptions had normal values but the numbers of cases are too small for median values to be calculated The expected correlation between

Table II *Degree of glucosuria at the time of the determination of the serum lipids*

| Control Glucosuria | No of pats |
|---|---|
| 0 | 83 |
| 1–20 g | 54 |
| 21–50 g | 32 |
| > 50 g | 14 |
| | 183 |

Table III *Form of therapy at the time of the determination of the serum lipids*

| Therapy | No of pats |
|---|---|
| Diet only | 25 |
| Insulin | 60 |
| Sulfonylurea | 34 |
| Phenformin | 32 |
| Sulf + phenformin | 29 |
| Insulin + phenformin | 4 |
| | 184 |

cholesterol values and age can hardly be said to be obvious with the method for the median used in this material

The distribution of the triglyceride values in different age groups is visualized in Fig 2 Eighty five of 184 patients had values of more than 150 mg and 64 had more than 200 mg One male patient 60 years of age had the extreme value of 6570 mg% and a cholesterol value of 960 mg% Lipid electrophoresis in this patient revealed an increase of chylomicrons and pre beta lipoprotein and classified the case as hyperlipoproteinemia type V according to Fredrichson et al (7) In this figure as well median values of the different age groups have been plotted using the sex symbols except for the infrequent younger cases The figure shows a trend to higher triglyceride values with higher age

Fig 3 gives the relation between cholesterol and fasting blood sugar The circles represent the mean values for males and females together at

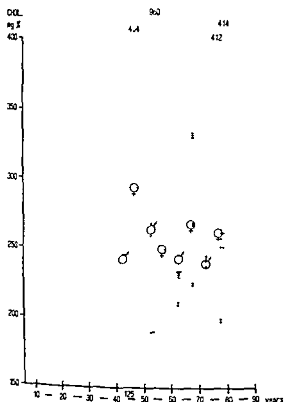



*Fig 1* Cholesterol values in male and female diabetics of different ages. The sex symbols represent the mean value of the age group

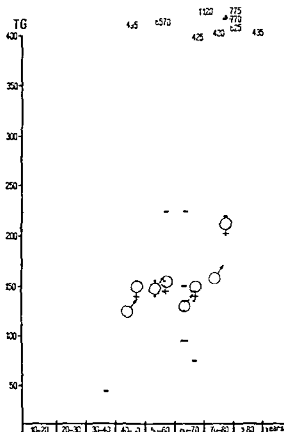



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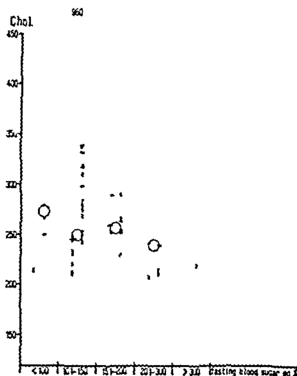



*Fig 3* Cholesterol values in relation to fasting blood sugar

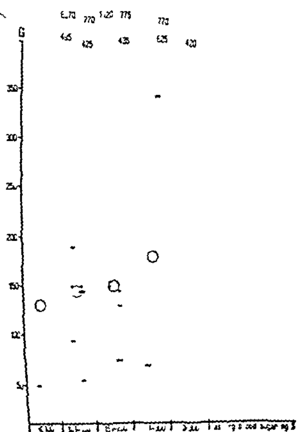



*Fig 4* Triglyceride values in relation to fasting blood sugar

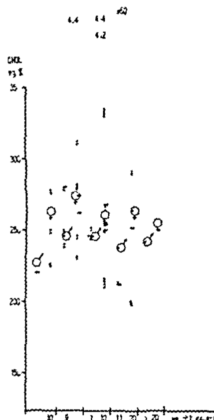


*Fig 5* Cholesterol values in relation to weight excess. On the abscissa is stated how far body weight exceeds body height in cm minus 100

triglycerides and weight excess is visualized in Fig 6 Here a distinct and even marked positive correlation is obvious

Attempts to study a possible relation between serum lipid valu s and duration of diabetes have not been made simply because we do not know the duration of diabetes in our patients In this clientele of diabetics fairly advanced in age we know rather exactly the time of diagnosis, but in elderly diabetics this is an entirely different matter from the time of the beginning of the disease which is quite impossible to decide afterwards

In 45 patients receiving Atromidin therapy it has been possible to determine lipid values before and during this medication These cases include the already mentioned patient with hyperlipoproteinemia type V Fig. 7 presents the effect of Atromidin on cholesterol values The figure on the left represents the mean value of three determinations made before and that on the right the mean value of three made during Atromidin therapy The average depression during therapy is

obvious Excluding the extreme case of hyperlipoproteinemia beyond the purpose of this investigation, the average depression in 44 cases is 46 7 mg

An even more remarkable decrease is seen in triglycerides (Fig 8) Here the average depression in 44 cases is 80 8 mg

It may be mentioned that we have seen no relevant side-effects with Atromidin in this material

## DISCUSSION

Previous authors (2 14) have found normal lipid values in treated diabetics of less than 30 years of age The present investigation includes only 14 patients in this age group but for these few cases the same is true with rare exceptions for both cholesterol and triglycerides Bierman et al (3) found increased triglyceride values in about one third of 40 adult men with a mildly abnormal glucose tolerance test These figures are in good agreement with our results 85 increased triglyceride values in 184 predominantly elderly diabetics

*Fig 6* Triglyceride values in relation to weight excess. On the abscissa is stated how far body weight exceeds body height in cm minus 100

*Fig 7* Cholesterol values before and during Atromidin therapy

The correlation found by certain authors (1 13) between triglycerides and fasting blood sugar corresponds to a slight such tendency in the present material On the other hand we have found no correlation between cholesterol and fasting blood sugar Like Bierman and Porte Jr (3) we have found a high percentage of increased triglyceride values in elderly diabetics in a good or fair diabetic control

Our material like those of many previous authors (3) shows an association between triglycerides and obesity but no such correlation between cholesterol and weight excess

Earlier reports of the effect of Atromidin on serum lipids in diabetics are scarce They have

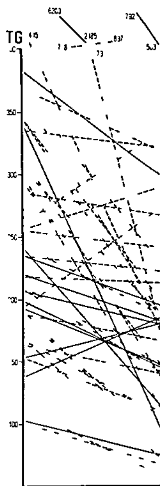


*Fig 8* Triglyceride values before and during Atromidin therapy

mostly concerned a few diabetics included in material of different diseases In these reports a decrease of the lipid values has been reported both in cholesterol (5 6 8 12) and in triglycerides (5)

Although as yet no conclusions can be drawn about the value of Atromidin in preventing complications and death of atherosclerotic disease the safety and the good lipid reducing effect presented in this material of 45 diabetics appear to justify an extended use in hyperlipemic diabetic patients Hood et al (9) among others recommended Atromidin therapy for individuals with a high risk of atherosclerotic complications Hyperlipemic diabetics seem to us to be such high risk individuals To be meaningful this lipid reducing therapy might be reserved for not too elderly patients e g. of less than 60 years of age

REFERENCES


1 Albrink, M J & Davidson, P C. J Lab clin. Med. 67 573 1966
2. Bierman E. L., Porte D Jr O Hara, D D., Schwartz, M L. & Wood F C., Jr J clin. Invest. 44 261 1965
3 Bierman E. L. & Porte D Jr Ann. intern. Med. 68 926 1968
4 Carlson L A. & Lindstedt S Acta med. scand. Suppl 493 1968
5 Danowski T S Novak J F Saul, R. W., Wester J W & Moses C Clin Pharmacol Ther 7 631 1966
6 De Mowbray R. R. J Atheroscler Res. 3 689 1963
7 Fredrichson D S Levy R. I & Lees, R. S Med. Progr (N Y ) 276 273 1967
8 Herriot S C Percy Robb I W Strong, J A. & Thomson, C G J Atheroscler Res. 3 679 1963
9 Hood B Angervall G Cramér K. & Welin G Acta med scand 184 337 1968
10 Kessler G & Lederer H. Technicon Symposia, p. 341 Mediad Inc New York 1965
11 Libermann C & Buchard, H In Technicon Symposia (ed J Levine S Morgenstern, & D Vlastelica) p 25 Mediad Inc New York 1967
12 Miller R D J Atheroscler Res 3 694 1963
13 Nikkila, E A. Pelkonen R. & Miettinen A. Diabetologia 2 232, 1966
14 Sterky G Larsson Y & Persson B Acta Paediat. 52 11 1963

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# AUTOANTIBODIES RELATED TO TREATMENT WITH CHLORTHALIDONE AND α METHYLDOPA

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*Abstract* Sera from 96 hypertensive patients and an equal number of matched controls have been studied for autoantibodies Significantly higher frequencies were found of antibodies to gastric parietal cells (14%) and antinuclear factors (A.N F) (14%) The direct anti human globulin (A.H G) test was positive in 5 of the hypertensive patients. No increase was found in the incidence of other autoantibodies. The occurrence of A.N F appeared to be related to treatment with chlorthalidone and the incidence of a positive direct A.H G test to α methyl dopa therapy A possible combined effect of these drugs on serological aberrations was observed. These aberrations appeared to be unrelated to the possible cause and severity of hypertension and to age and sex. No relation was found between treatment and antibodies to gastric parietal cells.

In 1966 Carstairs et al (2) suggested a relation between α methyldopa (Aldomet) therapy and haemolytic anaemia Further investigation revealed that although haemolytic anaemia is rarely seen in patients receiving α methyldopa, a positive direct antihuman globulin test on red cells (A H G test) was often present (1 3 9) The incidence of a positive A.H G test was found to be dependent on dosage and often became negative after withdrawal of the drug

Breckenridge et al (1) observed antinuclear factors (A N F) in 15% of sera from patients treated with α methyldopa and in 4% of hypertensive patients receiving other drugs The incidence of A.N F appeared to be unrelated to age to dosage of α methyldopa or to the presence of a positive direct A.H G test

In a preliminary report of a serological investigation of 145 hypertensive patients we confirmed the incidence of A N F in relation to α methyldopa no increase was found in the frequencies of other autoantibodies in patients receiving the drug (4) In the present investigation 96 of these 145 hypertensive patients were further examined and the relation between serological aberrations and drugs other than α methyldopa and their relation to sex age and cause and severity of hypertension were studied

## MATERIAL AND METHODS

*Patients*

Ninety-six patients with hypertension of varying degree and aetiology were studied. The diastolic blood pressure before treatment ranged from 90 to 1[illegible]0 most pressures were over 110 Before treatment 28 patients had grade III or IV (8) aberrations of the eye fundus due to hypertension. At the time of serological examination the patients had already received treatment for between 1 month and 10 years. Only 13 patients had a diastolic pressure of 110 or higher at the time while only three still showed residual haemorrhages of the eye fundus. None of the patients had clinical signs of systemic lupus erythematosus (S.L E) Normal subjects, matched for sex and age to the patients, were studied as controls

*Direct antihuman globulin test on red cells (A.H G test)*

The test was performed with sheep anti whole human serum according to standard methods, and in case of a positive reaction further specified with anti IgG anti IgM and anti-complement reagents. All antibodies proved to be of the IgG type

*Immunofluorescent technique*

The technique was performed as previously described (5) using a fluorescein-isothiocyanate conjugated IgG fraction of rabbit anti human immunoglobulin serum. All human sera were tested at a dilution of 1 10 except for the determination of antibodies to smooth muscle gastric parietal cells and mitochondria, which was performed with sera diluted 1 20

*Table I Incidence of autoantibodies related to chlorthalidone and α methyldopa therapy of hypertensive patients*

| | No | Pos dir A H G test | A N F (thyroid as substrate) | A N F pos with one or more substrates | Antibodies to gastric par cells |
|---|---|---|---|---|---|
| Total hypertensive patients | 96 | 5 (5 ) | 13[d] (14 ) | 23[d] (24 ) | 13[b] (14 ) |
| Matched normal controls | 96 | Not done | 0 | 2 (2 ) | 4 (4 ) |
| Treatment* | | | | | |
| Chlorthalidone | 56 | 5[a] (9 ) | 11[a] (20 %) | 20[c] (36 ) | 7 (12.5 ) |
| No chlorthalidone | 40 | 0 | 2 (5 ) | 3 (7 5 ) | 5 (12.5 ) |
| α methyldopa | 50 | 5[b] (10 ) | 9 (18 ) | 16[a] (32 ) | 5 (10 ) |
| No α methyldopa | 46 | 0 | 4 (9 ) | 7 (15 ) | 7 (15 ) |
| Both drugs | 32 | 5 (16 ) | 9 (27 ) | 15 (47 ) | 3 (9 ) |
| Chlorthalidone only | 24 | 0 | 2 (8 ) | 5 (21 ) | 4 (17 ) |
| α methyldopa only | 18 | 0 | 0 | 1 (6 ) | 2 (11 ) |
| None | 22 | 0 | 2 (9 ) | 2 (9 ) | 4 (18 ) |

* Irrespective of drugs other than chlorthalidone and α methyldopa
[a] $P<0\ 1$ [b] $P<0\ 05$ [c] $P<0\ 01$ [d] $P<0\ 001$

*L E cell test*

The test was performed by the indirect method as described by Kievits and Schuit (6)

*Rheumatoid factor*

This factor was determined with human 0 red cells sensitized with rabbit immunoglobulins (5 7)

## RESULTS

Sera from 96 hypertensive patients were examined by the indirect immunofluorescent technique for the presence of antibodies to salivary duct cells smooth muscle mitochondria skeletal muscle thymic myoid cells gastric pari tal cells thyroid colloid thyroid follicular epithelial cells and adrenal cortex

Antibodies to gastric parietal cells were significantly more frequent in sera from hypertensive patients than in sera from normal subjects matched for sex and age ($P<0\ 05$) (Table I) No antibodies to salivary duct c lls skeletal muscle thymic myoid cells or adrenocortex were demonstrated in the serum of any of the patients studied The other antibodies occurred with frequencies not significantly different from those in matched controls

The technique for detecting the above antibodies also revealed A N F if present Human thyroid adrenal and parotid gland rat diaphragm kidney and gastric mucosa and fowl thymus were therefore used as nuclear substrates Each substrate revealed significantly higher frequencies of A N F when compared to normal controls ($P<0\ 05$) ranging from 14% with adrenal or thyroid tissue as substrate to 5% when thymus was used A N F was found with one or more nuclear substrates in 24% of hypertensive patients (controls 2 % $P<0\ 001$) (Table I) None of the A.N F positive sera revealed L E cells by the indirect L.E -cell test

The direct A H G test was positive in five (5%) of the 96 blood samples (Table I) Only one out of five pati nts with a positive direct A H G test showed A N F with thyroid and two were positive for A N F with one or more substrates It was therefore deduced that the figures of these s rological aberrations were too small to demonstrate a correlation

A rheumatoid factor was demonstrated in only 3% of the sera and in none of matched controls

*Medication*

The 96 hypertensive patients were being treated with 34 different drugs chlorthalidone (Hygroton) to 56 patients α methyldopa (Aldomet) to 50 reserpine to 21 furosemide (Lasix) to six debrisoquinesulphate (D clinax) to five and chlordiazepoxide (Librium) to five patients. In addition 28 miscellaneous drugs were being used each for less than five patients. None of the patients were being treated with hydralazine a drug known to be related to S L.E.

Table II *Age and sex in relation to serological aberrations and drug therapy*

| | No | ♂ | ♀ | < 53 y | ≥ 53 y |
|---|---|---|---|---|---|
| Hypertensive patients | 96 | 50 | 46 | 47 | 49 |
| Pos dir A H G test | 5 | 1 | 4 | 2 | 3 |
| A N F (thyroid as substrate) | 13 | 8 | 5 | 5 | 8 |
| A N F (one or more substrates) | 23 | 12 | 11 | 10 | 13 |
| Antib to gastric par cells | 13 | 7 | 6 | 3 | 10 |
| Antib to thyroid colloid | 6 | 2 | 4 | 1 | 5 |
| Antib to thyroid cytoplasm | 4 | 0 | 4 | 2 | 2 |
| Receiving chlorthalidone | 56 | 27 | 29 | 23 | 33 |
| Receiving α methyldopa | 50 | 27 | 23 | 25 | 25 |
| Receiving both drugs | 32 | 17 | 15 | 13 | 19 |

$P < 0.05$

*Chlorthalidone and α methyldopa*

Patients treated with chlorthalidone or α methyldopa appeared to have significantly higher frequencies of serological aberrations compared to patients on other drugs No such relation was found for the other drugs

Table I shows that patients receiving chlorthalidone had a possibly higher incidence of positive direct A H G test ($P < 0.1$) and a significantly higher incidence ($P < 0.01$) of A N F compared to patients on other drugs The patients receiving α methyldopa had significantly higher frequency of the positive direct A H G test ($P < 0.05$) and possibly a greater incidence of A N F ($P < 0.1$) compared to patients on other drugs None of the other antibodies studied showed any significant relation to either drug

Within the group of 50 patients taking α methyldopa concurrent treatment with chlorthalidone was significantly related to an increase in the frequency of A N F when thyroid is used as substrate ($P < 0.05$) However within the group of 56 patients on chlorthalidone additional treatment with α methyldopa was not significantly related to an increased frequency of A N F with thyroid as substrate ($P < 0.1$) (Table I) Chlorthalidone treatment may therefore be more closely related to the incidence of A N F than is the administration of α methyldopa The number of patients on only one of these drugs is too small to be conclusive as five of 24 patients receiving chlorthalidone only had A N F as against one out of 18 on α methyldopa only

A combined effect is possible as the incidence of A N F is higher if both drugs are given than is to be expected from addition of the expected frequency for each drug ($P < 0.05$ for thyroid as substrate $P < 0.1$ positive with one or more substrates) Similarly a positive direct A H G test is related to the drug combination ($P < 0.05$) (Table I)

*Dose and duration*

As 41 out of 56 patients treated with chlorthalidone received 200 mg per week it was difficult to demonstrate any dependency of serological aberrations on dosage A positive direct A H G test was present in none of 14 patients on less than 200 mg during the last six months while of 42 patients receiving 200 mg or more five were positive for this test However these five patients re

Table III *Incidence of a positive direct A H G test and of A N F related to possible cause and severity of hypertension*

| Cause and severity of hypertension | No | Pos. dir A H G test | A N F (thyroid as substrate) | A N F (one or more substrates) | Antibodies to gastric par cells |
|---|---|---|---|---|---|
| Essential | 70 | 3 (4 ) | 8 (11 ) | 16 (23 ) | 9 (13 ) |
| Not essential | 26 | 2 (8 ) | 5 (19 ) | 7 (27 ) | 4 (15 ) |
| Chron glom nephr | 5 | 1 ( 0 ) | 1 (20 ) | 1 (20 ) | 0 |
| Chron pyelonephr | 6 | 0 | 2 (33 ) | 2 (33 ) | 1 (17 ) |
| Other chron nephr | 4 | 0 | 0 | 1 (25 ) | 1 (25 ) |
| Cong. polycystic kidneys | 2 | 0 | 0 | 0 | 0 |
| Renal artery stenosis | 2 | 1 (50 ) | 1 (50 ) | 1 (50 ) | 1 (50 ) |
| Miscellaneous | 7 | 0 | 1 (14 ) | 2 (29 ) | 1 (14 ) |
| Fundoscopic changes | | | | | |
| Grade I and II | 68 | 4 (6 ) | 8 (12 ) | 13 (19 ) | 8 (12 ) |
| Grade III and IV | 28 | 1 (4 ) | 5 (18 ) | 10 (36 ) | 5 (18 ) |

ceived both chlorthalidone and α methyldopa The incidence of A N F was not significantly related to the dose of chlorthalidone nor was the dosage of α methyldopa related to s rological aberrations in the present study

A positive direct A H G test was found in four out of 15 patients treated for more than two years with α methyldopa and in only one out of 35 patients treated for a shorter time ($P<0\ 05$) The same relation was found if the duration of treat ment with both drugs was considered None of the other serological aberrations was related to duration of chlorthalidone or α methyldopa ther apy

*Age and sex*

The figures presented in Table II demonstrate the absence of any influence of sex and age on the relation between drug therapy and serological aberrations as only antibodies to thyroid cytoplasm show d a preponderance of females ($P<0\ 05$)

*ause and severity of hypertension*

he hypertensive patients were grouped according to the probable cause of the hypertension (Table III) None of the groups revealed a significant preponderance of serological aberrations.

The severity of the hypertensive disease was assess-d by fundoscopic examination Haemor rhages exudates and/or papilloedema (grade III and IV) were found in 28 patients before or dur ing treatment while 68 patients had never shown these retinal changes. As shown in Table III there was a higher incidence of A.N F in the more severely affected group but the differenc is not significant ($0\ 2>P>0\ 1$)

At the time of the serological investigation only 13 pati-nts had a diastolic blood pressure of over 110 None of these had a positive A H G test two had A N F with thyroid and four A N F with one or more substrates Serological aberra tions were thus not found to be significantly re lated to blood pressure as measured at the time of investigation

Nine patients were not treated with chlorthali done owing to severly insufficient kidney func tion Only one of thes patients had a positive A N F This makes it less likely that s rological ab rrations are related to renal insufficiency per s-

## DISCUSSION

In the pres nt study the incidence of a positive direct A H G test was 10% in th pati nts treated with α methyldopa This figure is lower than the incidence of 20% reported by Carstairs et al (3) The discrepancy may possibly b ex plained by the lower dosage of the drug given to the patients in the present study (42% <1 g/day 36% 1–2 g/day 22% ≥2 g/day)

In a preliminary report (4) we mention d th incidence of A N F in 22% of 145 hyp rtensive patients This was significantly higher than the 2% in controls matched for sex and age Twenty eight % of hypertensive patients treated with α methyldopa had A N F If the occurrenc of A N F was related only to α methyldopa therapy an additional explanation would be required for the r latively high incidence (16 %) found in pa tients on other drugs which was still significantly higher than that in matched normal controls. The latter finding stimulated a further search for other factors responsible for serological aberrations. However no correlation was found with the possible cause of the hyp rtension or with the sex and age of the patients

As patients with more severe hypertension would be exp cted to have received more inten sive treatment we investigated the possibility that the serological abnormalities were related to th severity of the hypertension rather than to its treatment Although a higher incidence of A N F was found in the more severely hypertensive pa tients this correlation did not prove statistically significant

It appeared from the present study that treat ment with chlorthalidone may also be related to serological ab rrations. It is possible that chlor thalidone is even more closely related to the inci dence of A.N F than α methyldopa and a com bined effect of chlorthalidone and α methyldopa might be of even greater importance Further study would however be necessary to confirm these sugg stions

Th- present study gives no indication whether the drugs involved might have an effect on red cells and nuclear antig ns leading to alterations provoking the formation of antibodies cross-react

ing with unaltered auto-antigens or whether these drugs have a more direct influence on the behaviour of the immune system Further clinical and experimental work is required in order to answer these fundamental questions

## ACKNOWLEDGEMENT

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## REFERENCES

1 Breckenridge A Dollery C T Worlledge S M Holborow E. J & Johnson, G D Positive direct Coombs tests and antinuclear factor in patients treated with methyldopa. Lancet 2 1265 1967

Carstairs, K. C., Worlledge S M., Dollery C T & Breckenridge A. Methyldopa and haemolytic anaemia. Lancet I 201 1966

3 Carstairs, K. C Breckenridge A. Dollery C T & Worlledge S M Incidence of positive direct Coombs test in patients on alpha methyldopa. Lancet 2 133 1966

4 Feltkamp T E W Engelfriet C P & van Loghem J J Autoantibodies and methyldopa. Lancet I 644 1968

5 Feltkamp T E. W & van Rossum, A L. Antibodies to salivary duct cells, and other autoantibodies, in patients with Sjogren s syndrome and other idiopathic autoimmune diseases. Clin. exp Immunol. 3 1 1968

6 Kievits J H. & Schuit, H R. E. A simple indirect L E cell test with increased sensitivity Vox Sang. (Basel) 2 288 1957

7 Van Loghem Langereis, P E. Onderzoek van methoden naar de agglutinatie bevorderende factor in sera van patienten met rheumatoide arthritis. Bull Centr Lab Bloedtransf Dienst Ned Roode Kruis 2 230 1952.

8 Scheie H G Evaluation of ophthalmoscopic changes of hypertension and arteriolar sclerosis. A.M.A. Archives of Opthalmology 49 117 1953

9 Worlledge S M Carstairs, K. C. & Dacie J V Autoimmune haemolytic anaemia associated with α methyldopa therapy Lancet 2 135 1966

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# EXPERIENCES WITH TWO SIMPLE ASPIRATION LIVER BIOPSY TECHNIQUES

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*Abstract* Two simple aspiration liver biopsy techniques, suitable in clinical routine work, are described. These techniques, employed in more than 500 patients, yielded material sufficient for histological examination in all but occasional cases One fatal bleeding occurred and in one patient an arteriovenous intrahepatic aneurysm was dis co ered some years after liver biopsy

The clinical value of percutaneous needle biopsy of the liver in the diagnosis of liver disease is generally accepted Since Menghini introduced the one second needle biopsy technique (7) this method has gained world wide usage There is however one technical difficulty in the perform ance of this type of liver biopsy—the operator has to aspirate by retracting the plunger with one hand at the same time as the cannula is advanced into the liver parenchyma with the other These opposite movements of the hands may cause un intentional movements of the needle thus increas ing the risk of complications This difficulty can be overcome by a two-man technique one opera tor aspirating with a syringe connected to the cannula by a tube and the other introducing the cannula into the liver (8)

In the present paper two other simple aspiration biopsy techniques are described The first method (I) is based on that of Radner (10) The second (II) is based on that described by Franzen et al (4) for cytological fine needle aspiration biopsy

## METHODS

Only patients without a history of bleeding tendency and with normal coagulation time bleeding time and a platelet count above 100 000 were accepted for biopsy Patients found not to be able to control their respiration as instructed were not biopsied. Biopsy was performed by the intercostal technique after local anaesthesia with the patient in supine position as described by Sherlock (11)

### *Method I*

*The biopsy instru ent* (Figs. 1 and )

In order to obtain material for certain chemical analysis (liver iron determination) needles with an internal diameter of 1 6 mm (outer diameter [illegible]0 mm) and a length of 10–16 cm were used The needle is sharpened so that the tip is oblique forming an angle with the length axis of the needle of slightly less than 45 degrees, and with the cutting be el on the internal edge The needle should be sharpened after every second biopsy For routine diagnostic purposes thinner needles were used.

The needle is connected to a 10 ml syringe via a one way stop-cock The plunger stem of the syringe carries a stop in order to keep the plunger in locked aspirating position after retraction. Needles, stop-cocks and syringes are of standard type It is important to choose needles with thin walls (not above 0 [illegible] mm) It is technically simple to sharpen the needles and to supply the plunger stem with a stop Otherwise syringes are commercially available (Kifa, Stockholm, Sweden) As the needles are of standard type a great number can be kept available at negligible cost.

*Procedure*

With two to three ml of sterile saline solution in the syringe and the stop-cock in open position the needle is inserted through a small skin incision down to the inter costal space One ml of the saline solution is injected to clear the needle and to fill it with fluid The stop-cock le er is now turned to the closed position and the plunger is retracted. The plunger is kept in aspirating pos tion by the plunger stop The patient is told to breathe out and then to hold his breath Now aspiration is achieved by turning the stop-cock le er to the open pos tion and the needle is rapidly advanced 3–5 cm into the liver without rotation and then rapidly extracted The stop-cock is closed before the needle is wholly extracted (unless a Menghini needle is used which is supplied with an inter nal stoppage) to pre ent the biopsy specimen from being

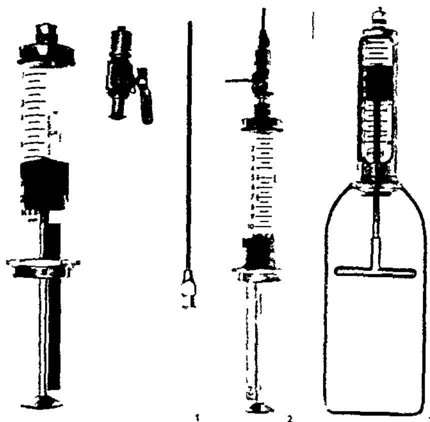



*Fig 1* Photographic reproduction of the instrument in method I

*Fig* The instrument in method I ready for biopsy The plunger is kept in aspirating position by the stop

*Fig 3* Photographic reproduction of the Franzén syringe used in method II

aspirated into the syringe After extraction of the needle the plunger is released from its aspirating position, the stop-cock is opened and the biopsy cylinder is carefully expelled

## Method II

*The biopsy instrument* (Fig. 3)

The syringe used was devised by Franzen (4) for cytological fine needle aspiration biopsy Aspiration with this syringe is done with one hand Menghini needles (supplied with Luer locks) with an outer diameter of 1 2 mm were mostly used. The rear of the Menghini needle is supplied with an internal block allowing fluid passage but preventing the biopsy specimen from being aspirated into the syringe (7)

*Procedure*

With two to three ml of sterile saline solution in the syringe the needle is introduced down into the intercostal space where one ml of the solution is injected With the patient holding his breath (after expiration) the plunger is maximally retracted and the needle is rapidly introduced 3-5 cm into the liver parenchyma and then rapidly extracted still during maintained aspiration. After extraction of the needle the aspiration is broken off and the biopsy specimen is carefully expelled directly into the fixative

## RESULTS

More than 500 biopsies have been performed by the described techniques When using thin needles (outer diameter 1 2–1 4 mm) the two methods are quite comparable and sufficient material for histological examination was obtained in 94% of the cases In advanced cirrhosis of the liver the material obtained however was often small and fragmented

Thick needles (internal diameter 1 6 mm and external diameter 2 0 mm) and method I were used in a series of 105 patients in order to obtain material both for histological examination and for chemical analysis of liver iron In this series sufficient material for histological examination was obtained in all but four subjects and three fourths of the specimens were large enough for chemical analysis The mean dry weight of the specimen used for chemical analysis was approximately 10 mg

## COMPLICATIONS

Many patients had pain in the right hypochondrium and sometimes referred pain in the right

shoulder region The pain was usually mild and of short duration In a few patients however analgesics were given

In one patient fatal bleeding occurred a 57 year-old man with aetiologically obscure cardio megaly and azotaemia high sedimentation rate and increased gamma globulin fraction The liver was enlarged Liver function tests indicated active liver disease serum glutamic oxalacetic trans aminase was 330 units/ml and glutamic pyruvic transaminase 270 units/ml (normal values less than 40 units) Serum bilirubin was borderline and serum alkaline phosphatases 18–34 units (n v less than 10 units) Liver biopsy was performed with a Menghini needle with an outer diameter of 1 2 mm No respiratory movement was noted dur ing the biopsy and there were no immediate com plications The patient suddenly died eight hours after the biopsy without preceding blood pressure fall Increased heart rate was present already be fore biopsy At autopsy 1 2 l of blood mixed fluid containing blood clots was found in the abdominal cavity The liver was considerably enlarged and had an increased consistency On the lateral sur face of the right liver lobe a 2 8 cm long sagittal rift was found and was considered to be the cause of the bleeding Histologically vascular changes of the type found in primary amyloidosis could be demonstrated in all organs studied

In one woman born in 1907 liver biopsy was performed in 1951 with the Iversen Roholm in strument In February 1965 she was biopsied by the present technique I because of abnormal liver function tests The outer diameter of the needle was 2 mm The biopsy was easily performed and there were no immediate postbiopsy complica tions Study of the biopsy specimen revealed slight steatosis After discharge the condition was essen tially unchanged In April 1967 a strong contin uous murmur over the liver was accidentally detected Aortography showed an arteriovenous aneurysm of plum size in the right liver lobe The localization of the aneurysm was consistent with the possibility that it was caused by transthoracic liver biopsy The aneurysm was drained by en larged hepatic veins

Clubbing of the fingers and prominence of the pulmonary artery arc (in the absence of heart murmur) had been noted as early as 1950 A pulmonary angiography performed in 1965 (be fore the second liver biopsy) showed dilated central arteries and reduced flow through the pul monary vessels This case will be reported in detail elsewhere (1)

## COMMENT

Needles with an outer diameter of 1 2–1 4 mm are easily introduced into the liver parenchyma and with such needles the biopsy is extremely simple to perform with the aspiration syringe de vised by Franzen et al (4) In clinical routine work we now use Menghini needles (outer diam eter 1 2–1 4 mm) combined with the Franzen syringe The Franzen syringe is easily handled with one hand and the needle is in the liver for less than one s cond Complications are consid ered to be less with the Menghini needle than with the Vim Silverman needle (5 6) The mor tality risk is estimated to be considerably less than 1/1000 Nevertheless one fatal bleeding occurred after biopsy with a Menghini needle In this patient liver biopsy was considered risky because of heart decompensation and was therefore per formed with a fine needle (outer diameter 1 2 mm) At autopsy a rift was found in the liver indicating that the patient made a respiratory movement during the biopsy The underlying dis ease was found to be primary amyloidosis and the liver was congested One fatal bleeding after liver biopsy in a patient with amyloidosis was reported by Volwiler and Jones (15) and in creased danger of postbiopsy bleeding was con sidered to be present in this disease (14) Biopsy in liver congestion has also been reported to be associated with increased bleeding complications (13) However amyloidosis or liver congestion are not considered by most operators using the Men ghini needle to contraindicate biopsy (5) It is commonly stated that the frequency of complica tions increases with increasing needle thickness (7) If however the patient makes a respiratory movement during biopsy the rifting effect of a thin stiff needle might be more pronounced than that of a thick needle

We use thick needles if large material is required i e for chemical analysis or if cirrhosis of the liver is suspected In advanced cirrhosis of the liver we found it difficult to obtain enough material with thin needles for histopathological interpretation With thick needles we have good experiences with the technique first described

(Method I) However in one woman, biopsied in 1951 and in 1965 with thick needles, an intrahepatic arteriovenous aneurysm was diagnosed in 1967. Though several arteriovenous intrarenal fistulas following renal biopsy have been reported (for ref. see Smith et al. (12)) so far only two cases of intrahepatic arteriovenous fistula after percutaneous liver biopsy have been reported (3, 9). In those patients the fistulas were hepatoportal. In the present patient the aneurysm was drained into dilated hepatic veins.

A few cases of intrahepatic arteriovenous aneurysms without relation to biopsy or trauma have also been reported. Most of them were children and they also had other vascular malformations (for ref. see Childers et al. (2)). The two adults reported had Osler's disease and in one of them pulmonary vascular obstruction was diagnosed (2). In our patient a diagnosis of Osler's disease could not be established, but a pulmonary angiography performed in 1965 (before the second liver biopsy) showed peripheral pulmonary vascular constriction. Furthermore, clubbing of fingers and prominence of the pulmonary artery arc were noted as early as 1950 (before the first liver biopsy). Thus there is some evidence that the pulmonary vascular changes, which might have been secondary to the aneurysm, may have been present already before the first biopsy. It cannot be excluded, however, that the aneurysm was a consequence of liver biopsy. Increased attention should be focused on the possibility of such aneurysm as complication of liver biopsy.

## ACKNOWLEDGEMENT

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## REFERENCES

1. [illegible], G. & D[illegible], O. A case of intrahepatic arteriovenous aneurysm. To be published.
2. Childers, R. W., Ranniger, K. & Rabinowitz, M. Intrahepatic arteriovenous fistula with pulmonary vascular obstruction in Osler-Rendu-Weber disease. Amer. J. Med. 43, [illegible], 1967.
3. D[illegible], C., L[illegible], J., Martin, E., Hernandez, C., C[illegible], J. & Conte, F. Fistules artério-veineuses hépato-portales consécutive à une ponction-biopsie du foie. Découverte artériographique. Presse méd. 76, [illegible], 1968.
4. Franzén, S., Giertz, G. & Zajicek, J. Cytological diagnosis of prostatic tumours by transrectal aspiration biopsy. A preliminary report. Brit. J. Urol. 32, 193, 1960.
5. Lindner, H. Grenzen und Gefahren der perkutanen Leberbiopsie mit der Menghini-Nadel. Erfahrungen bei [illegible] Leberbiopsien. Dtsch. med. Wschr. [illegible], 1967.
6. M[illegible], R. Risiko der Leberbiopsie. Helv. med. Acta [illegible], 1963.
7. Menghini, G. One-second needle biopsy of the liver. Gastroenterology 35, 190, 1958.
8. Menghini, G. Two-operator needle biopsy of the liver. A new, easier and safer version of the one-second technic. Amer. J. dig. Dis. 4, [illegible], 1959.
9. Preger, L. Hepatic arteriovenous fistula after percutaneous liver biopsy. Amer. J. Roentgenol. 101, 619, 1967.
10. Ra[illegible], S. Another type of biopsy needle. Scand. J. clin. Lab. Invest. 14, [illegible], 1962.
11. Sherlock, S. Diseases of the Liver and Biliary System. Ed. 3. Blackwell, Oxford 1963.
12. Smith, G. H., Jr, Remmers, A. R., Jr, Dailey, B. M. & Sarles, H. E. Intrarenal arteriovenous fistula and systemic hypertension following percutaneous renal biopsy. Report of a case. Nephron [illegible], 1968.
13. S[illegible], M. H. Needle biopsy of the liver. Surg. Clin. N. Amer. 47, [illegible], 1967.
14. Stauffer, M. H., Gross, J. B., Foulk, W. T. & Dahlin, D. C. Amyloidosis: diagnosis with needle biopsy of the liver in eighteen patients. Gastroenterology 41, [illegible], 1961.
15. Volwiler, W. & Jones, C. M. The diagnostic and therapeutic value of liver biopsies. With particular reference to trocar biopsy. New Engl. J. Med. 237, 651, 1947.

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# VON WILLEBRAND S DISEASE IN AN ICELANDIC FAMILY

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*Abstract* In a family with von Willebrand s disease thir teen members, eight males and five females, had a history of excessive bleeding. These are from a total of 41 indi viduals in nine pertinent sibships. Severe symptoms of bleeding predominate in the males, two of whom died from haemorrhage Typical lesions of hereditary haemor rhagic telangiectasia were found in one female who is considered to be a carrier of the von Willebrand s disease gene There is a reduced expressivity of the mutant gene amounting to nonpenetrance in at least some of the female members of the family

The laboratory clinical and hereditary findings in this family suggest much higher prevalence of the mutant gene than is indicated by clinically affected cases.

In addition to the classical criteria of the heredi tary pattern prolonged bleeding time and vascular abnormality the diagnosis of von Willebrand s disease has been made more precise by the estima tion of factor VIII level (1 14 17 20) and by studying the reaction of affected individuals after infusion of plasma or certain plasma fractions (7 4) A further improvement in diagnosis has also been made by the separation of thrombasthenia from von Willebrand s disease by the use of ADP in the thrombocyte aggregation test (10)

Agreement on the usefulness and role of tests for assessing platelet adhesiveness in von Wille brand s disease has not been reached (16 22)

As no direct, reliable test is available to ascer tain the presence and type of gene or genes caus ing von Willebrand s disease in every case the problem of genetic heterogeneity is unresolved when one is dealing with unrelated families or individuals diagnosed as suffering from von Wille brand s disease This problem can be overcome whenever opportunity permits by investigating several related family units with affected members In such an investigation it is easier to asso iate the well known variability of this disorder with the same gene or genes

Apart from the classical studies in Finland by von Willebrand (23) and Jurgens et al (14) ex tending over three decades notable family and laboratory studies of this disease in Scandinavia have been made in Sweden (14 15)

In this paper studies are presented on the first reported cases of von Willebrand s disease in Ice land

## MATERIAL AND METHODS

*Bleeding time* The method of Ivy (13) was used on all but two members of the family seen. Duke s (8) method was carried out in parallel on four of the members, whilst this was the most convenient method to use on one child VI 2.

*Whole blood coagulation t me* (WBCT) The method was based on that of Lee and White (15) One ml vol of blood was delivered into 8 mm internal bore glass tubes previously warmed to 37 C.

For other coagulation tests, blood was drawn in plast c syringes and mixed in the proportion 9 1 with 3 2 tri sodium citrate dihydrate and centrifuged for 20 min at 1500 *g* to obtain platelet poor plasma.

*One stage p othromb n time* (PT) was determined using rabb t brain thromboplastin

*Partial th omboplast n ti ne with kaolin* (PTTK) was determined by mixing 0 1 ml vol of plasma with 0 1 ml ol of kaolin suspension, 5 mg/ml in buffer pH 7 2 and incubating the mixture for 10 min at 37 C The reaction was completed by adding 0 2 ml vol of a mixture in equal proportions of Bell and Alton l poid (2) and 0 025 M calcium chloride solution.

*Th omboplastin gene at on t sts* were performed as de scribed by Biggs and Douglas (3) except that Bell and Alton lipo d (2) was used as platelet substitute

*Facto VIII assay* In most cases a one-stage technique based on the PTTK (10) was used with Inosthin as the phospholipid. For the infusion studies on IV 21 the two-stage method of Pitney (19) based on the thromboplastin

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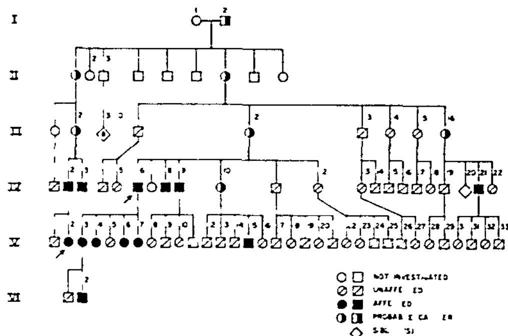



*Fig. 1.* Pedigree of the family.

generation test was carried out using bovine factor V (Diagnostic Reagents Ltd.) instead of absorbed haemophilic plasma. The "normal" 100% standard was from of us (L. W.) who has been shown previously to a value about the mean of a pool of normal plasma les.

*Platelet counts* were performed by the method of Brecher and Cronkite (5).

*Platelet aggregation tests* were carried out as described by Hardisty and Ingram (10).

*Hess tests.* The patient's arm was inspected for the presence of petechiae. A sphygmomanometer cuff was placed round the upper arm and inflated to a pressure of 80 mm of mercury and this was maintained for 5 min. After this time the pressure was released and, when the circulation had returned to normal, the volar aspect of the forearm was inspected for any further petechial haemorrhages.

*Infusion studies.* Cryoprecipitate was prepared by Dr Valtyr Bjarnason at the Blood Bank of Iceland using the slow method of Pool and Shannon (21). The concentrates, which were free of red cells, were injected by syringe within half an hour of reconstitution and took less than five min to administer.

## The Pedigree Studies and Family Survey

The relationship in the von Willebrand's disease family is shown on the pedigree drawing (Fig. 1).

The common ancestor of the family was born in 1822. It is therefore probable that the disease causing mutant gene common to all the affected members had been present in the family for nearly 150 years, extending over six generations.

The daughter of the progenitor II 1 and her Icelandic husband migrated to Canada, and later their grandchildren moved to California and thus form the American part of this family.

The survey of relatives embraces 1?0 individuals belonging to generations III to VI. Of these a total of 41 individuals belonged to nine sibships which all contained one or more affected siblings or had one affected parent. The average size of sibships was approximately 4.6.

Diagnosis has been based mainly on clinical data, supplemented by coagulation studies in 17 patients, ten of whom manifested a bleeding tendency.

### Notes on the cases

I 2. Born in 1822, died 1877. Known to have a bleeding tendency. Was in Holstein in Denmark when war broke out between Denmark and Germany. He cut his leg and kept the wound open to avoid being called up and he bled excessively.

II 1. Went to America. No information available.

II. 7. Bled excessively after a tooth extraction and had to be hospitalized.

III 3–10. Siblings contain one female baby who died at three months from persistent haemorrhage from the umbilicus. The sibship contains a male who has "bruises of long duration" and whose son at the age of 15–16 bled excessively after tooth extraction, but his other ten children have had no signs of a bleeding tendency.

III 12. Born in 189?. Has hereditary haemorrhagic telangiectasia. Had bleeding episodes into the gastrointestinal tract nine years ago and again repeatedly in the last 3–4 years. Has 2–4 telangiectatic spots in the oral cavity. Diagnosis of a typical telangiectasia of the gastric mucosa was made by gastric camera (Dr H. Jonasson).

III 11, 13, 14, 15. No symptoms.

III 16. A woman aged 52 years when she was operated on because of an intracranial aneurysm. She bled exces-

sively postoperatively and had to be operated on again. She became haemiplegic and died five years later

IV 1 No symptoms of bleeding

IV 2 Born in 1917 died in 1958 Bled a lot from minor wounds as a child In 1939 at the age of 22 he bled profusely after a tooth extraction and *had to be* given a blood transfusion at the University of California Hospital He was diagnosed as haemophilia His daughter was later found to have von Willebrand's disease cf case V 2 At age 24 he was hospitalized after walking into a wooden packing box. During 1957 he was found to have kidney stones but because of his bleeding tendency was not operated upon In the latter part of that year he began to have haematuria and was repeatedly transfused He died in January 1958 aged 41

IV 3 When aged nearly three years he was injured by a sharp stone thrown by a small boy The cut on his forehead bled excessively and the wound was dressed by a doctor The bleeding appeared to have stopped but two or three days later his head appeared swollen and he died that day

IV 4 5 No signs of bleedings.

IV 6 Born in 1917 He had repeated haemorrhages from the kidney and intestinal tract Bleeds excessively from small wounds Bleeding time (Duke) has been prolonged (15 min) when he has been in a bleeding phase in hospital

IV 7 Died at an early age from an infectious disease

IV 8 Died at the age of [illegible]0 from persistent intestinal haemorrhages Bled excessively from scalp cuts and after tooth extractions. Once he bled into the knee joint Was in hospital repeatedly because of his bleeding tendency For the last four years of his life he was in hospital suffering from intestinal haemorrhage from which he died Telangiectasia? — cf his mother III 12

IV 9 Born in 19 2. Aged 46 Bleeds excessively from minor wounds and tooth extraction (up to three days) He has suffered from haematuria on several occasions once connected with exertion once provoked by taking sulpha drugs because of febrile illness and once when he had influenza. He sometimes gets extensive bruises from minor traumata

IV 10–12 No symptoms of bleeding

IV 13 Aged 30 Excessive bleeding from cuts At age of [illegible]0 she had two teeth removed and bled excessively At age 25 many teeth extracted without trouble Bruises easily

IV 14–19 No symptoms referable to bleeding tendency

IV [illegible]0 Died at an early age

IV [illegible]1 Aged 32 years. Symptoms of excessive bleeding appeared at age 15–[illegible]0 Bled into thigh muscle when playing football Kidney haemorrhages on three occasions after being confined to bed for several days recovering from appendectomy when he had febrile illness and following a running competition shortly after a course of sulpha drugs

IV [illegible]2. Age 30 years No bleeding symptoms He has four children with no symptoms

V 1 Unaffected

V 2 Aged 19 years Severe bleeding following tooth extraction requiring hospital treatment at Stanford Medical Center in California Was investigated by Dr J G Pool

Table I *Results of coagulation tests*

| | Normal | III 12 | IV 6 | IV 9 (Bjarni) | IV 10 | IV 13 | IV 21 (Sverrir) | V 3 | V 4 | V 5 | V 6 | V 7 | V 11 | V 15 | VI 1 | VI 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tourniquet test (Hess) | Neg | Weak +ve | Neg | Neg | Neg | Neg | Neg | Weak +ve | Neg | Neg | Neg | Neg | Neg | Neg | Neg | — |
| BT (Ivy) | 9 min | 6½ | 5½ | 3 | 3 | *11* | 3 | 4½ | 4½ | 4½ | 4½ | 3 | 3 | 4 | — | — |
| BT (Duke) | 6 min | — | 5 | — | — | — | 2 | 3 | 5 | — | — | — | — | — | 3 | — |
| WBCT | 10 min | — | 8 | 6 | 4 | — | 7½ | 4½ | 6 | 6 | 7 | 7 | 6 | 9 | 4½ | — |
| One stag prothrombin | 11 14 s c | 13 | 13 | 12 | 12 | 12 | 12 | 13 | 13½ | 13 | 12½ | 12½ | 13 | 12 | 12 | 12 |
| PTTK | 39–46 s c | 37 | *65* | *65* | 38 | 38 | *51* | 44 | 52 | 50 | *63* | *64* | 43 | *53* | 41 | 54 |
| Factor VIII | 50–200 | — | *30* | *15* | 60 | — | *10* | *30* | 100 | 50 | *30* | *22* | 70 | *10* | 85 | *15* |
| Platelets | 150–400 100 per mm | — | 230 | 250 | — | — | — | — | 320 | 241 | 220 | 233 | 220 | — | — | — |

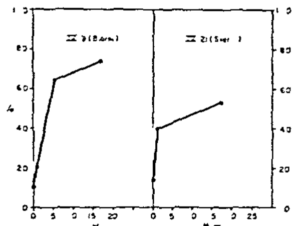



*Fig 2.* Disproportionate rise of factor VIII in two family members following infusion of factor VIII concentrate

whose tests showed a prolonged bleeding time and a low level of factor VIII Furthermore studies showed a disproportionate rise of factor VIII and a diagnosis of von Willebrand's disease was made

V 3 Age 27 years. Normal clinically

V 4 Age 23 years. Bruises easily Has had menorrhagia. She has also bled severely after tooth extraction.

V 5 Bruises easily Age 20 years.

V 6 Age 16 Complains of bruising.

V 7 Age 12 years. Complains of bruising.

V 8–11 Ages 24 18 15 and 12 years, respectively No

V 12, 13 14 16 Ages 23 19 15 6 No symptoms.

V 15 Age 13 years. Has had severe bleeding from tooth extractions and bleeding from minor wounds.

V 17 33 No symptoms.

VI 1 No symptoms.

VI 2. Age 2 years. Excessive bleeding when mother cleaned ears and when he bit his lip

## RESULTS

The results of the bleeding times measured by the Ivy method in 13 members of this family are shown in Table I These members were selected on the basis of availability and were not all clinically affected. Bleeding times were normal in all subjects although borderline in IV 13 Likewise the bleeding times measured by the Duke method in five individuals were normal in all cases

The Hess test was performed on 14 individuals and was clearly normal in all individuals tested Thrombocyte aggregation was found to be normal in two patients tested.

During the course of this study the normal control plasma gave a range of values in the PTTK test from 39 to 46 sec. Assuming that 7 sec or greater prolongation represents an abnormality in this test seven out of 15 gave an abnormal result.

Plasma factor VIII concentrations were 50% or less in eight of 13 subjects tested Only four of the nine however showed values of 15% or less

There was quite a good correlation between the PTTK time and the factor VIII assay All factor VIII levels below 30% showed a prolongation greater than 7 sec in the PTTK test although one individual with 30% factor VIII gave a time only 5 sec longer than the control

### Infusion studies

Cryoprecipitate was administered to two subjects to assess whether they were able to synthesize factor VIII as a result of this stimulus

Subject IV 9 was given 20 ml of cryoprecipitate in which the factor VIII was concentrated ×7 The results of factor VIII assays are shown in Fig. 2 Immediately after infusion the plasma factor VIII content rose from 10 to 20% However evidence of new synthesis of factor VIII was obtained from the samples at 5 and 17 hours, in which the factor VIII content was assayed at 65 and 75% respectively Similar results are shown in Fig. 2 for subject IV 21

Patient V 2 and her brother were investigated by Dr Pool at Stanford University School of Medicine The tests showed a prolonged bleeding time and a low level of factor VIII Infusion studies showed a disproportionate rise of factor VIII and a diagnosis of von Willebrand's disease was made Her brother V 1 was found to be normal.

Clinical manifestations of a bleeding tendency were found in 13 individuals, five females and eight males. Two males have died because of bleeding, namely IV 3 IV 8 and possibly also case IV 2 (see clinical histories) The history of

Table II *History of bleeding symptoms and factor VIII levels in asymptomatic phase*

| Subjects | Nos | History of bleeding | Factor VIII (%) | Pedigree no |
|---|---|---|---|---|
| Males | 4 | | 10–15 | IV 9 IV 1 V 15 VI |
| | 1 | | 30 | IV 6 |
| Females | 2 | – | 30–50 | V 3 V 5 |
| | 2 | | 20–30 | V 6 V 7 |
| | 1 | – | 100 | V 4 |

bleeding symptoms and factor VIII levels in ten affected subjects when asymptomatic is shown in Table II

## DISCUSSION

It is commonly accepted that patients with a normal bleeding time and a low factor VIII level may be classified as mild haemophiliacs On the other hand for a diagnosis of von Willebrand s disease recent criteria have be n a long bleeding time and a deficiency of factor VIII with a Mendelian dominant inheritance affecting both sexes *As in haemophilia, a negative family history does* not exclude the diagnosis but unlike haemophilia the degree of severity may vary considerably within a family Also it is well known that the bleeding time and the clinical severity may vary from time to time in any given patient

In the present survey of a family in which the inheritance and sex incidence was in favour of the diagnosis of von Willebrand s disease most of the affected patients although giving histories of prolonged bleeding times had normal bleeding times at the time of testing and on the basis of laboratory studies alone a wrong diagnosis of haemophilia could have been made In two such patients the prolonged response to the intravenous administration of cryoprecipitate pointed unmistakably to von Willebrand s disease

As seen from the case notes the most common symptoms of bleeding in both sexes were superficial bruises variable in size provoked by small insults and often not accounted for Excessive bleeding from minor cuts and wounds was more frequent in the males mainly due to small hazards at work Five males and two females gave histories of prolonged bleeding following tooth extraction One male is known to have bled into the knee joint and another into his thigh muscles while playing football

The more serious episodes of internal bleeding, such as from the urinary and gastrointestinal tract are with one exception to be discussed confined to the males in the family and have led to death in two cases

Buchanan and Leavell (6) analysed the symptoms in von Willebrand s disease in 199 cases 95 males and 104 females assembled from the medical literature They found upper gastrointestinal haemorrhage to be more frequent in males (16 or 17° ) than in females (6 or 6 ) and haematuria was also more frequent in males (7 5° ) than females (2 ₒ)

The presence of the gene of von Willebrand s disease is indicated by overt symptoms of bleeding in 13 family members eight males and five females 28 oth r individuals in the nine sibships are free from symptoms Four females are considered to be carriers of the mutant gene namely III 12 III 16 IV 10 and V 3 These are all mothers of affected sons who have suffered from excessive bleeding (see Fig 1 and clinical notes) Case V 3 has had no symptoms of bleeding She had a normal bleeding time and Hess test *but a factor VIII* level of 30° Her cousin VI 10 was found to have a factor VIII level of 60 ₒ and no symptoms of bleeding at any time She may possibly be taken as an example of nonmanifestation of the mutant gene Her mother III 12 had no signs of excessive bleeding until the age of 60 when she was admitted to hospital because of severe gastrointestinal haemorrhage At the age of 70 she again experienced troublesome episodes of intestinal bleeding At that time typical lesions in the stomach of hereditary haemorrhagic telangiectasia were found with the aid of a gastric camera The occurrence of these two conditions in the same patient is a rare and interesting phenomenon and has been described previously by Horler and Witts (12) but not explained Despite her vascular disorder she had a normal bleeding time and Hess test and her factor VIII level was considered to be normal as she had a PTTK time below the normal control Her second son IV 8 died at the age of 20 from severe and chronic intestinal bleeding (see case notes) Her first son IV 6 has been severely affected repeatedly (case notes) he had a factor VIII level of 30% at the time of investigation the same value as his first daughter who has however no symptoms His second daughter on the other hand V 4 showed no abnormality in the coagulation tests (factor VIII 100 %) but has had severe menorrhagia and increased bruising

In the original von Willebrand s disease families the females were more prone to severe symptoms and death from bleeding (23) The reverse is a notable feature in this family Symptoms both of excessive bleeding and fatal haemorrhage are more pronounced in the males For this reason the disorder had previously been wrongly diagnosed as haemophilia in this family

From Fig. 1 it can be seen that examples of transmission from father to son are lacking This could well be fortuitous because the chances are few This along with the reasons named above has probably also been misleading in making the right diagnosis in the past.

More probable carriers of the mutant gene can be detected if asymptomatic siblings parents and other close relatives are ascertained by estimating their factor VIII level (17) Although providing valuable evidence of the presence of the mutant gene the difficulties of ascertainment are not solved by this parameter alone due to other causes of variation and testing error The subnormal values of factor VIII found in many clinically unaffected family members investigated by these authors strongly suggest a much higher prevalence of the mutant gene than is indicated by the number of clinically affected cases The deviation from the expected genetic ratio in the family presented and the marked sex differences already discussed likewise suggest the possibility that the abnormal gene or genes exist in many family members in a latent form

As stated the observations recorded have been while selected family members were in good condition (Table II) With the exception Dr Pool s case V 2 who had a factor VIII of 35% while in the bleeding phase no factor VIII estimations have been made on the other family members when they had bleeding symptoms Pitney and Arnold (20) stated that factor VIII concentration is relatively constant in von Willebrand s disease and is independent of fluctuation in vascular response or clinical episodes

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REFERENCES

1 Alexander B & Goldstein, R *Dual hemostatic* defect in pseudohemophilia J clin Invest 32 551 1953
2 Bell, W N & Alton, H G A brain extract as a substitute for platelet suspension in the thromboplastin generation test Nature (Lond.) 174 880 1954
3 Biggs, R & Douglas, A S The thromboplastin generation test. J clin. Path. 6 23 1953
4 Biggs, R & Matthews, J M The treatment of haemorrhage in von Willebrand s disease and the blood level of factor VIII (AHG) Brit J Haemat. 9 203 1963
5 Brecher G & Cronkite E P Morphology and enumeration of human blood platelets. J appl. Physiol. 3 365 1950
6 Buchanan, J C & Laevell, B S Pseudohemophilia report of 13 new cases and statistical review of previously reported cases. Ann intern Med 44 241 1956
7 Cornu, P Larrieu, M D., Caen, J & Bernard, J Transfusion studies in von Willebrand s disease effects on bleeding time and factor VIII Brit. J Haemat. 9 189 1963
8 Duke W W The pathogenesis of purpura haemorrhagica with especial reference to the part played by the blood platelets. Arch intern. Med 10 445 1912
9 Hardisty R. M., Dormandy K. M & Hutton, R A Thrombasthenia studies on three cases. Brit. J Haemat. 10 371 1964
10 Hardisty R. M & Ingram, G I M *Bleeding disorders Blackwell, Oxford 1965*
11 Hardisty R. M & Macpherson J C A one-stage factor VIII (antihaemophilic globulin) assay and its use on venous and capillary plasma. Thromb Diath. Haem. (Stuttg.) 7 215 1962
12 Horler A. R. & Witts L. J Hereditary capillary purpura. Quart. J Med 27 173 1958
13 Ivy A. C Nelson D & Bucher G The standardization of certain factors in the cutaneous venostasis bleeding time technique J Lab clin Med. 26 1812, 1940
14 Jurgens R., Lehmann W Vegelius O Eriksson, A. W & Hupler E Mitteilung uber den Mangel an antihamophilem Globulin (Faktor VIII) bei der Aalandischen Thrombopathie (v Willebrand Jurgens) Thromb Diath haem. (Stuttg.) 1 257 1957
15 Lee R. I & White P D A clinical study of the coagulation time of blood. Amer J med Sci 145 495 1913
16 MacFarlane, R. G International review of experimental pathology vol 6 p 119 Academic Press, New York and London 1968
17 Nilsson, I M Blomback M & von Francken, I On an inherited autosomal hemorrhagic diathesis with antihemophilic globulin (AHG) deficiency and prolonged bleeding time Acta med scand 159 35 1957
18 Nilsson I M Blomback, M Jorpes E Blomback, B & Johannsson, S von Willebrand s disease and its correction with human plasma Fraction I-0 Acta med scand. 159 179 1957
19 Pitney W R. The assay of antihaemophilic globulin (AHG) in plasma. Brit J Haemat. 2 250 1956
20 *Pitney W R. & Arnold, B J Plasma antihaemo*philic factor (AHG) concentrations in families of patients with haemorrhagic states Brit J Haemat. 5 184 1959
21 Pool, J G & Shannon A. E Production of high-potency concentrates of antihemophilic globulin in a closed-bag system. New Engl. J Med 273 1443 1965
22 Salzmann F W Measurement of platelet adhesiveness. J Lab clin. Med. 62 724 1963
23 von Willebrand E. A. Uber hereditare Pseudohamophilie Acta med. scand. 26 521 1931

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# EXTREME PERSISTENT EOSINOPHILIA WITH HIGH SERUM $B_{12}$ VALUES

*A Report of Two Cases*

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*Abstract* A report is given on two male patients—35 and 47 years old—with pronounced eosinophilia of long duration The first patient is still alive after six years observation, while the second patient—a diabetic—died after four years because of liver cirrhosis with portal hypertension and repeated haematemeses

In both cases, as well as in four cases of persistent eosinophilia reported by other authors, serum vitamin $B_{12}$ was considerably elevated. It is discussed whether this finding may be interpreted as supporting a leukaemic nature of the diseases To elucidate this theory further we made a pilot test on twenty cases of eosinophilia of wellknown origin which all showed normal serum B concentrations.

In the literature on eosinophilic leukaemia serum $B_{12}$ determinations are mentioned only exceptionally It therefore must be emphasized that serum $B_{12}$ should be incorporated in the laboratory investigation programme when eosinophilia is present.

Blood eosinophilia is a feature in many diseases above all in allergic states and parasitic infections Also collagen diseases—panarteritis nodosa in particular—can bring about an overproduction of eosinophilic granulocytes The same is true of Hodgkin s disease and other states of malignancy An eosinophilic response is seen also in relation to certain intoxications and skin lesions All these secondary eosinophilias may present leukaemoid features eosinophilia however disappears when the underlying disorder—if possible—is cured or controlled

Many cases of eosinophilia are only slight and transitory (13) so that the nature of the reaction is never established In the majority of patients with pronounced eosinophilia however one of the above mentioned diseases can be demonstrated

Occasionally no explanation for the eosinophilia can be found Dittrich s (4) collection of such cases illustrates the complexity of the group Many cases have been reported as eosinophilic leukaemias but there is no general agreement on the justification of this term On one side Evans and Nesbit (7) and Bentley et al (1) advanced criteria for the disease On the other side Bousser (2) displayed much scepticism referring among others to Engfeldt and Zetterstrom (6) these authors proposed the term disseminated eosinophilic collagen disease on the theory that immunity mechanisms cause most of the so-called eosinophilic leukaemias The discussion has been resumed by Odeberg (16) by Karle and Videbæk (12) and most recently by Hardy and Anderson (11) who prefer the collective term the hypereosinophilic syndromes This stands for a continuum of closely related diseases with innumerable intermediate forms and sharply defined subgrouping is not meaningful according to the authors

The aim of this study is to report in detail on two patients with persisting eosinophilia In addition we want to draw attention to the elevated serum concentrations of vitamin $B_1$ in our patients and in a few other reported cases The importance of this observation has not been discussed in this connection before it may be interpreted as supporting a leukaemic nature of the diseases Backing up this view we have examined serum $B_1$ in a group of secondary eosinophilias which all show normal values

## CASE REPORTS

*Case 1*

A 35 year-old previously healthy carpenter was admitted to Glostrup Hospital on August 5 1963 because of pain

Table I *Differential counts on bone marrow aspirations and peripheral blood (case 1)*

| Date of examination | July 12 1963 | | Nov 1 1963 | | June 6 1966 | | March 25 1969 |
|---|---|---|---|---|---|---|---|
| Specimen examined | Marrow | Blood | Marrow | Blood | Marrow | Blood | Blood |
| Haemocytoblasts | 1 5 | | | | 1 | | |
| Promyelocytes | 4 5 | | 0 5 | | 3 | | |
| Neutroph myelocytes | 17 5 | 2 | 9 | | 13 | | |
| Eosin myelocytes | 18 5 | | 18 | | 15 | | |
| Metamyelocytes | 7 | 2 | } 27 | | 13 | | 3 |
| Neutr band forms | 6 | 1 | | 4 | 6 | 2 | 1 |
| Polymorphonucl leuk | 14 | 24 | 18 | 37 | 13 | 35 | 28 |
| Eosinophilic leukocytes | 12 5 | 58 | 21 | 40 | 17 | 33 | 38 |
| Basophilic leukocytes | | | | | | | |
| Lymphocytes | 14 | 9 | 2 | 19 | 3 | 28 | 28 |
| Monocytes | | 4 | | | | 2 | 2 |
| Plasma cells | 0 5 | | | | | | |
| Reticulum cells | | | 0 5 | | | | |
| Megakaryocytes | | | 0 5 | | | | |
| Basophil erythroblasts | | | 0 5 | | 1 | | |
| Polychrom erythroblasts | 1 | | 1 | | 5 | | |
| Eosinophil erythroblasts | 3 | | 2 | | 10 | | |
| Basophil megaloblasts | | | | | | | |
| Eos and polychrom meg bl | | | | | | | |
| White blood cell count/µl | | 32 000 | | 14 700 | | 7700 | 11 00 |

of three weeks duration in the left gluteal muscle following a game of football There was a family history without evidence of blood disorders allergic or malignant

The physical examination showed an apparently healthy man The only abnormal findings were that the liver reached 4 cm below the right costal border and that small lymph nodes could be felt in the groins

*The laboratory data on admission were* Hb 12 g/100 ml ESR 103–62 mm/h WBC 15 500–40 200/µl Differential count showed 53–78 eosinophilic cells Eosinophilic cell count in peripheral blood 12 900–26 400/µl Reticulocytes 1 2–1 8/100 erythrocytes No haemolytic factors (Coombs test Ham and Crosby s test and Donath Landsteiner reaction were negative no warm haemolysins) Platelets 180 000/µl Serum iron transferrin and folic acid normal Serum vitamin B 7500 pg/ml Schilling test normal intestinal absorption of vitamin $B_{12}$ Bone marrow (aspirated by sternal puncture) dominance of eosinophilic cells and a myelopoiesis with a slight shift to the left The eosinophilic cells in the peripheral blood showed abnormal features hypersegmentation of nuclei and vacuoles in the cytoplasm (Bone marrow aspirations and differential counts from the peripheral blood are listed in greater detail in Table I) Liver function was normal according to the ordinary laboratory tests Microscopic examination of liver tissue (needle biopsy a m Menghini) normal structure but many eosinophilic cells in the sinusoids In the periportal tissue several infiltrations with polymorphonuclear and eosinophilic cells were found in relation to blood vessels Fractioned serum protein (by paper electrophoresis) normal Serum creatinine and urine examinations normal The serum reaction for toxoplasmosis was negative Faeces gave negative reaction with benzidine and no parasites or ova were found microscopically Histological *examination of two* lymph nodes from the groin showed unspecific chronic inflammation with many eosinophilic cells Skin and striated muscle were normal microscopically Examination of smear from colon mucosa showed no inflammation no eosinophilia X ray *examinations were normal* (chest stomach intestines cholegraphy pelvic bone and lumbar vertebrae) Ophthalmoscopic examination normal no leukaemic infiltrates

The broad spectrum anti parasitic drug dithiazanin was given in an attempt to exclude a *primary parasitic infection* however eosinophilia was not reduced Also prednisone treatment was tried in a dose of 40 mg per day but was withdrawn after three weeks as the eosinophilia did not show any decrease The patient s moderate fatigue did not diminish either during the *treatment and further* more itching of the skin started in this period The slight normochrome anaemia responded well to iron which was given perorally for two months When discharged on September 7 1963 the patient was in subjective well being

During admission for a check up in November 1963 treatment with busulphan (Myleran) was started with 4 mg per day It was reduced to 2 mg per day four weeks later After one year we reduced the dose further to ? mg every second day because of low platelet counts, which finally led to withdrawal of Myleran in April 1965 (platelets 30 000/µl) The untraditional Myleran treatment (Myleran continuously during one year and a half instead of administration for only short periods as usual in leukaemia therapy) was essayed due to the fact that—although complete haematological remission did not occur—the leucocyte count persisted normal during treatment, haemoglobin was almost normal and the disorder as a whole seemed to be under control

We have followed the patient for six years now He is

Table II *Differential counts on bone marrow aspirations and peripheral blood (cas 2)*

| *Date of examinat on* | *Sept 13 1965* | | *June 7 1966* | | *Febr 14 1967* | | *July 18 1968* | |
|---|---|---|---|---|---|---|---|---|
| Specimen examined | Marrow | Blood | Marrow | Blood | Marrow | Blood | Marrow | Blood |
| Haemocytoblasts | 2 | | 1 | | 0 5 | | 2 | |
| Promyelocytes | 7 | | 2 | | 1 5 | | 9 | |
| Neutroph myelocytes | 2 | | 3 | | 9 | | 12 | |
| Eosin myelocytes | 16 | | 10 | | 1[illegible]5 | | 8 | |
| Metamyelocytes | 12 | | | | 8 5 | | 14 | |
| Neutr band forms | 2 | 1 | | 10 | 0 5 | | | 9 |
| Polymorphonucl leuk | 4 | 8 | 8 | 18 | 3 5 | 30 | 4 | 19 |
| Eosinophilic leukocytes | 27 | 80 | 41 | 59 | 17 5 | 45 | 8 | 37 |
| Basophilic l ukocyt s | | 2 | | | 0 5 | | 0 5 | |
| Lymphocytes | 5 | 8 | 5 | 11 | 13 | 25 | 15 | 48 |
| Monocytes | | 1 | | 2 | | | 0 5 | 7 |
| Plasma cells | 1 | | | | 0 5 | | 1 | |
| Reticulum cells | | | | | | | 2 | |
| Megakaryocytes | | | | | | | | |
| Basoph l erythroblasts | 2 | | 1 | | 2.5 | | | |
| Polychrom. erythroblasts | 6 | | 11 | | 12 5 | | 4 | |
| Eosinophil erythroblasts | 14 | | 18 | | 17 | | | |
| Basophil megaloblasts | | | | | | | | |
| Eos. and polychrom meg bl | | | | | | | | |
| White blood cell count $\mu l$ | | 11 400 | | 4000 | | 1900 | | 3300 |

well nourished, does not feel ill and works full-time in his profession. He has had a short period with transient paresthesias in the left arm and occasionally a feeling of thoracic oppression but never pulmonary infiltrates on X ray examination. During the last four years he has been treated with diphenhydramine 25 mg per day because of the persisting tendency to itching of the skin. Hb is normalized, 14 9 g/100 ml. WBC 3400 11[illegible]00 $\mu l$ with 71–51 % eosinophilic cells. Eosinophilic counts 16[illegible]5–4025/$\mu l$ blood Platelets 150 000–3[illegible]0 000/$\mu l$. ESR 47–8 mm/h. Serum vitamin $B_{12}$ conc 2760–9100 pg ml. Serum LDH normal

The chromosomes in the leucocytes from a bon mar row specimen in June 1966 were examined by Bent Pedersen, M D (University Institute of Medical Genetics, Copenhagen) Neither the Philadelphia chromosome nor other chromosome abnormalities could be demonstrated.

*Case 2*

A 47 year-old male floor planer was admitted to Glostrup Hospital on September 6 1965 because of eosinophilia. He had had diabetes, treated with insulin, since the age of 28 The last year before admission he experienced a great loss of weight (10 kg) fatigue intermittent upper abdominal pain, itching of the skin and occasionally cramp in the sole muscles. In addition se eral bouts of hypoglycaemia had occurred. No family history of blood diso ders, allergic or malignant diseases existed.

On examination the patient appeared pale slightly emaciated with non-tender hepatosplenomegaly but no palpable lymph nodes. The skin presented several scratch marks. Ophthalmoscopy showed diabetic retinopathy

*Labo atory results on admission* Hb 13 g/100 ml. ESR 35 mm h. WBC 14 000–8000/$\mu l$ blood with 70–80% eosinophilic cells and no immature cells by differential count. Eosinophilic cell count 3160–9050 $\mu l$ blood and platelet count 150 000 $\mu l$ blood Sternal puncture aspi rate hyperplastic bone marrow with dominance of eosinophilic leucocytes and unaffected erythropoiesis see Table II for further details. Serum iron and transferrin normal, bleeding time and coagulation time normal Serum vitamin $B_{12}$ conc 3900–11 800 pg ml Serum bilirubin 1.2–1 8 mg/100 ml. Alkaline phosphatase 30 K.A. units (norm. 3–10) Serum protein 6 8 g 100 ml with low albumin and high gammaglobulin, both 2.3 g/100 ml by paper electrophoresis. Prothrombin 56% of normal (Owren Aas) Serum GO and GP transaminases and acid phosphatase also normal. Needle biopsy a.m. Menghini from li er portal cirrhosis and subacute-subchronic pericholangitis with many eosinophilic cells. Serum creatinine and urine examinations normal except glucosuria. No parasites or ova on repeated examinations of faeces. Microscopy of lymph node from left axilla unspecif c inflammation with eosinophils X ray examinations showed splenomegaly and oesophageal aricosities. Elimination of contrast by intravenous cholegraphy not visible Colon stomach and chest were normal.

Eosinophilia showed no decrease during one week with prednisone treatment 40 mg per day and white blood cell count and symptoms (fatigue anorexia, itching) remained unaffected. Next we started with busulphan (Myleran) 6 mg per day soon reduced to 2 mg per day since it did not bring haematological remission e ther When discharged from hospital on October 17 1965 the patient received per day Myleran 2 mg, prednisone 7.5 mg, and insulin. This continued until Feb 1967 except for two periods where Myleran was withdrawn because of thrombocytopenia and neutropenia respectively Myleran (2 mg per day) however was given again since withdrawal of the cytostatic drug had no beneficial effect o

Table III *Serum $B_{12}$ in secondary eosinophilia*

| | Eosinophilic cell count in peripheral blood per μl | Sex and age of the pats | Concentrations of vitamin $B_{12}$ in serum |
|---|---|---|---|
| Thirteen pats with asthma bronchiale | 600–800 | ♀ 29 ♂ 82 | 260 and 330 pg/ml |
| | 801–1000 | ♀ 21 ♀ 33 ♀ 64 ♂ 65 | 350 820 400 and 430 pg/ml |
| | 1001–1200 | ♀ 56 ♂ 58 | 140 and 510 pg/ml |
| | 1201–1500 | ♀ 21 ♂ 28 ♀ 51 ♀ 55 | 420 410 480 and 250 pg/ml |
| | 2502 | ♀ 31 | 600 pg/ml |
| Four pats with eosinophilic dyspnoea | 956 | ♀ 40 | 360 pg/ml |
| | 1069 | ♀ 53 | 410 pg/ml |
| | Max. 2945 | ♀ 44 | 460 pg/ml |
| | Max. 6750 | ♂ 33 | 516 pg/ml |
| One pat with Hodgkin's disease | Max. 1131 | ♂ 43 | 250 pg/ml |
| One pat with Hodgkin's disease earlier operated for adenocarcinoma recti | Max. 7188 | ♂ 60 | 2 0 pg/ml |
| One pat with collagen disease (?) | Max. 1775 | ♂ 61 | 280 pg/ml |

mentioned cytopenias. Although the patient's complaints remained unaltered he was on the whole able to do his work.

Myleran was completely withdrawn in February 1967 because of serious depression of the bone marrow Hb g 100 ml WBC 1900/μl with 4–59 partly hyper ted eosinophilic cells, but no immature features in other leucocytes. Platelets, 14 000/μl blood. The patient was re admitted to receive blood transfusions This, however was complicated by incompatibility reactions, and he was still anaemic on discharge His ESR, bilirubin alkaline phosphatase and plasma protein had remained unaltered except from a slight decrease in the gamma globulin fraction in serum Anti nuclear factors could not be demonstrated. The serum vitamin $B_{12}$-concentrations had ranged from 1030–5280 in the preceding period.

We raised the prednisone to 15 mg per day and in the following year he improved gradually There were Hb 11–12 g/100 ml and normal white blood cell counts, but still eosinophilic counts 2100–3500 μl Platelet counts remained low (max. 85 000/μl) The general improvement persisted, also after reduction of prednisone which was completely withdrawn in April 1968

In May 1968 an abscess on the left calf entailed treatment in a surgical department.

On June 14 1968 a new phase began. He was admitted with haematemesis presumably due to bleeding from oesophageal varicosities He was transferred to the Surgical Department S of Gentofte Hospital with the purpose of estimating whether a portacaval shunt should be made The contraindications, however were considered too strong. (*a*) haemorrhagic diathesis due to thrombocytopenia and low values of coagulation factors II and VII refractory to intravenous vitamin K administration (*b*) blood incompatibility difficulties and (*c*) an infected wound on the left leg. His ESR was 25 mm h, Hb 7.6–10.4 g 100 ml WBC 2960/μl, eosinophilic count 1363/μl blood platelets 50–75 000/μl Sternal puncture aspirate showed hyperplastic normoblastic erythropoiesis and a myelopoiesis with a shift to the left and some eosinophilia. Serological tests for haemolysis were negative Serum B 5616 pg/ml. Prothrombin 38 alkaline phosphatase 37 K.A. units normal serum bilirubin, serum transaminases and galactose elimination Gamma globulin 20 g l by serum paper electrophoresis Liver biopsy (a.m. Menghini) *portobiliar spaces a little broader than usual* with acute and chronic moderately eosinophilic inflammatory reaction. He was discharged on August 1 1968 with menadione and insulin only

The dominance of the hepatic component in the composite picture continued in the patient's last months the persisting eosinophilia now seemed of minor importance He was readmitted to Glostrup Hospit l on October 5 1968 with severe haematemesis which did not respond to haemostatic treatment with Sengstaken Tube vitamin K and numerous fresh blood transfusions. Ascites and slight icterus developed too Four weeks later he was again transferred to Gentofte Hospital Dept S X ray splenoportography confirmed the liver cirrhosis with portal hypertension and varicosities in stomach and oesophagus. A portacaval anastomosis was made on vital indication. The postoperative course was complicated by atony of stomach and sepsis with Staphylococcus aureus He died on December 6 1968 in hepatic coma. 18 days after the operation

*Autopsy* did not show leukaemic infiltrates There was hepatic cirrhosis with steatosis and stasis of blood and bile In the hyperplastic spleen and the at ph c pancreas eosinophilia could not be demonstrated microscopically The same was true of kidneys, skin tissue and skeletal muscle Some eosinophilia however could still be demonstrated in lymph nodes and bone marrow The heart (250 g) showed no abnormalities in peri myo- and endo-

cardium. Disseminated bronchopneumonias were seen in the lungs.

Finally we may point out that the Philadelphia chromosome could not be demonstrated in this case either. The bone marrow cells were examined by Bent Pedersen, M.D., in June 1966, i.e. early in the clinical course.

The pathological departments at Glostrup and Gentofte performed the microscopic evaluations of tissues and bone marrow.

### *Own Examinations*

*Serum $B_{12}$ in secondary eosinophilia*

In order to establish the nature of the above two cases we performed a pilot test on the serum vitamin $B_{12}$ concentrations in eosinophilia of well known origin. We collected 20 such patients whose data are recorded in Table III. The diagnoses are seen in the left column. There were no significantly elevated values of serum $B_{12}$ (determined with Lactobacillus Leishmanii) and no correlation of the vitamin concentration to the eosinophilic cell counts. Thus the tendency in the pilot test is quite clear, but of course it is desirable that larger groups should be examined.

## DISCUSSION

The controversial aspects of eosinophilic leukaemia (EL) have been outlined in the introduction. Several cases of eosinophilia display leukaemic features and many authors consider it reasonable to regard EL as a disease entity. Bentley et al. (1) analysed twenty cases, which were subdivided into two groups with immature and mature eosinophilic cells respectively. In the latter group the clinical course could be either stormy with myeloblastic transformation or chronic and protracted.

Our two cases might fit into Bentley's last group. They could not be classified among the usual secondary eosinophilias. Allergy and collagen disease were not probable since steroids apparently had no effect and specific pathological lesions were absent in numerous biopsy microscopies. Eosinophilia was continuously high in peripheral blood and bone marrow except in the terminal phase of case 2. Eosinophilic infiltrates were demonstrated in hepatic tissue and lymph nodes.

The above considerations, however, do not throw new light on the problems concerning EL. We therefore want to draw attention to the abnormally high serum $B_{12}$ concentrations in the two patients: 2760–9100 pg/ml and 3900–11 800 pg/ml respectively. Karle and Videbæk's (12) case of EL displayed a serum $B_{12}$ conc. of 3500 pg/ml and Odeberg's (16) two patients presented elevated values too (2900 and 1100 pg/ml). Another case with an EL-like picture, ending in myeloblastic crisis, has been reported (20); the 48 year old man showed serum $B_{12}$: 2240 pg/ml. We have not found serum $B_{12}$ specifications in other reports on EL.

The Lactobacillus Leishmanii method has been used for vitamin $B_{12}$ determinations in our patients. The values from serum of normal patients are 125–720 pg $B_{12}$/ml (8). According to the literature abnormally high values of serum $B_{12}$ occur in three groups of patients:

1) In some patients receiving vitamin $B_{12}$ therapy due to pernicious anaemia (22).

2) In patients suffering from liver diseases with damage of hepatic cells (25, 26).

3) In chronic myelogenous leukaemia (CML). Fischer (8) found 1160–20 700 pg $B_{12}$/ml serum with an average of 5300 pg/ml in this group. 91 % of the patients had concentrations above 2000 pg/ml. Abnormally high values of serum $B_{12}$ is a more inconstant finding in acute myelogenous leukaemia (8, 23), while other myeloproliferative diseases usually show quite normal values.

Our patients apparently belong to the last of the three groups, but the liver cirrhosis theoretically might contribute to the high serum $B_{12}$ in case 2. This, however, is not probable according to Østergard Kristensen's findings (27): a marked but transitory serum $B_{12}$ elevation occurred only in patients with acute hepatitis, while the serum values in chronic hepatitis and cirrhosis ranged from 320–945 pg/ml.

Schwartz and Bastrup-Madsen (22) showed that the vitamin $B_{12}$ in CML was bound to the same proteins (transcobalamine I and II) in serum as the endogenous vitamin $B_{12}$ in normal persons; the abnormally high capacity for $B_{12}$ binding, however, was not saturated more than 40–60 %. Miller and Sullivan (15) accordingly found the absolute binding capacity for added vitamin $B_{12}$ greatly increased in CML. This could not be reproduced in our patients. Their preliminary high serum $B_{12}$ concentrations did not alter significantly during a three month period with massive $B_{12}$ administration (7 injections i.m. of 1 mg cyanocobalamine (Betolvex) every two weeks in the summer of 1966).

Finally we must refer to our pilot test on serum

$B_{12}$ in secondary eosinophilia, showing normal values of the vitamin.

Marked elevations of serum vitamin $B_{12}$ in otherwise leukaemia-suspected haematological disorders consequently favour the diagnosis *chronic* myelogenous leukaemia. As for EL with high serum $B_{12}$ it could be suggested that it is in reality CML—only with preponderance of eosinophilic cells. Our two cases however—showing normal chromosome pattern of bone marrow cells without Philadelphia chromsome—differ from CML in this respect. According to Pedersen (18) the Philadelphia chromosome is a very constant feature in the bone marrow cells in CML, almost specific for the disease. In other reports on patients with EL (9, 10, 12, 21) bone marrow showed similar absence of the abnormal chromosome. This could be interpreted as support for regarding EL as a distinct entity.

## REFERENCES


1. Bentley, H. P., Reardon, A. E. & Knoedler, J. P. Amer. J. Med. 30: 310, 1961.
2. Bousser, J. Sang 28: 553, 1957.
3. Chen, H. P. & Smith, H. S. Ann. intern. Med. 52: 1343, 1960.
4. Dittrich, H. Acta haemat. 7: 230, 195_.
5. Engbæk, H. C., Heerup, L. & Thomsen, S. Nord. Med. 14: 1535, 1942.
6. Engfeldt, B. & Zetterstrom, R. Acta med. scand. 153: 337, 1956.
7. Evans, T. S. & Nesbit, R. R. Blood 4: 603, 1949.
8. Fischer, E. Ugeskr. Læg. 1_8: 8_0, 1966.
9. Goh, K., Swisher, S. N. & Rosenberg, C. A. Ann. intern. Med. 62: 80, 1965.
10. Gruenwald, H., Kiossoglou, K. A., Mitus, W. J. & Dameshek, W. Amer. J. Med. 39: 1003, 1965.
11. Hardy, W. R. & Anderson, R. E. Ann. intern. Med. 68: 12_0, 1968.
12. Karle, H. & Videbæk, Aa. Dan. med. Bull. 13: 41, 1966.
13. Laverdre, J., Lafontaine, E. & Courillon, J. Presse med. 74: 1077, 1966.
14. Mikkonen, L., Finkler, A. E. & Hall, C. A. Acta med. scand. 181: 591, 1967.
15. Miller, A. & Sullivan, J. F. J. clin. Invest. 38: _135, 1959.
16. Odeberg, B. Acta med. scand. 177: 129, 1965.
17. Papageorgiou, A. Blut 8: 338, 1962.
18. Pedersen, B. Ugeskr. Læg. 129: 1289, 1967.
19. Piney, A. Rev. Hemat. 4: 3, 19_9.
20. Rigshospitalet, Copenhagen, Denmark. Clinical pathological Staff Meeting, October 17, 1968.
21. Sandberg, A. A., Ishihara, T., Crosswhite, L. H. & Hauschka, T. S. Blood 20: 393, 1962.
22. Schwartz, M. & Bastrup-Madsen, P. Scand. J. Haemat. 5: 35, 1968.
23. Ståhlberg, K. G., Olsson, L., Gahrton, G. & Norden, A. Acta med. scand. 174: 105, 1963.
24. Thomsen, S. & Plum, P. Acta med. scand. 101: 116, 1939.
25. Whitby, L. E. H. & Britton, C. J. C. Disorders of the blood, 9th ed., pp. 176–177, 287, 547. Churchill, London 1963.
26. Wintrobe, M. M. Clinical hematology, 6th ed., p. 1031. Lea & Febiger, Philadelphia 1967.
27. Østergård Kristensen, H. P. Vitamin $B_{12}$ in human blood, pp. 10_–113. Thesis. Copenhagen 1958.

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# EXERCISE LEUKOCYTOSIS WITH AND WITHOUT BETA ADRENERGIC BLOCKADE

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*Abst act* Exercise leukocytosis during standardized work has been demonstrated in eight male healthy test subjects. Mostly the leukocytosis was due to an increase in segmented neutrophils, to a minor degree an increase in lymphocytes. After propranolol (Inderal®) administration exercise results in practically no increase in neu trophil leukocytes or in lymphocytes Exercise leukocytosis is concluded—at least partly—to be mediated by a beta receptor mechanism.

As early as 1893 it was shown that muscular work resulted in blood leukocytosis (11) In spite of numerous investigations since then exercise leukocytosis do s not appear to have been studied under standardized conditions until lately (1 2) In these studies it was demonstrat d that exercis leukocytosis is correlated to work intensity and duration Figures as high as 60 000 leukocytes/mm³ and above were reported Apparently exer cise leukocytosis is released by an adrenergic mechanism Other humoral factors in blood such as AHG (3 5) and fibrinogen (5) have been found to increase during exercise This increase is blocked by propranolol (5) With the aid of this selectively beta blocking agent propranolol (Inde ral®) we wished to study to what extent exercis leukocytosis is mediated by a beta receptor mech anism

## MATERIAL AND METHODS

The test subjects were eight healthy young male volun teers. According to Swedish standards they were all about mean he ght and weight. Two of the subjects were a little above average the remainder of ordinary physical f tness. Some data for the subjects are shown in Table I

A *health history* was recorded and a *physical examina t on* performed. *Height* and *weight* were measured. *Hae moglob n* concentration was determined in capillary samples accord ng to the oxyhaemoglobin method The *physical working capacity* was expressed as the amount of work that the subject could perform on a *bicycle ergometer* (9) at a pulse rate of 170 beats/min, W " (12) The same bicycle ergometer with unchanged static dimens ons were used for the same individual at both work tests. All *pulse rates* were calculated from electrocardiograms, at least 20 heart cycles being counted for each value The *number of leukocytes* was counted in capillary samples, and differential counts were made (2) the latter however only in six subjects (nos. 1 2, 3 5 6 and 7)

### *Expe imental p ocedure*

On two consecutive days a W ₇₀ test was performed. The test continued to maximal effort Samples for leukocyte counts were taken after 10 min of supine rest after 8 min of standing and at the end of each work load (6 min) as well as 10 min after the end of exercise Differential counts were made at the end of the resting period, at work load 600 kpm/min and at the end of exercise

On day 2 pulse rate leukocyte count and differential count were taken after the resting period Then 5 mg propranolol (Inderal®) was given intravenously during 5 min and pulse rate and leukocyte and differential count were taken again. The $W_{70}$ test was then per formed in the same way as on the day before

The dose of 5 mg propranolol is presumed to cause a satisfactory beta adrenergic blockade in man (10) With this dose there is no effect on the heart rate in the supine pos tion, as caused by the intravenous administration of 0 008 mg iso-proterenol.

## RESULTS

The results are shown in Tables II III and IV and Fig 1

During exercise without beta adrenergic block ade there was a marked rise both in puls rate

Table I *Some anthropometric and other data in eight test subjects*

| | Age (y) | Height (cm) | Weight (kg) | Haemoglobin (g/100 ml) | $W_{170}$ (kpm/min) |
|---|---|---|---|---|---|
| $\bar{x}$ | 23 4 | 181 5 | 72 9 | 14 1 | 1169 |
| S D | 0 7 | 6 8 | 8 1 | 1 2 | 101 |
| Range | 22–24 | 170–190 | 59–86 | 12 2–15 5 | 800–1700 |

and leukocyte count The mean work time was 29 6 min The final exercise mean pulse rate was 190 4 beats/min The corresponding leukocyte count was 14 460 leukocytes/mm³ blood 67 6 % being segmented neutrophils and 25 2 % lymphocytes The comparable resting values were 6480 leukocytes/mm³ 58 3 % neutrophils and 33 1 % lymphocytes respectively The final exercise l u kocyte count and the neutrophil part of it were significantly higher ($p<0\,001$) than at rest as also was the final exercise mean lymphocyte part ($p<0\,001$)

After beta blockade with propranolol the mean work time was 27 1 min The final exercise mean pulse rate was 158 1 beats/min The puls rate at rest and 10 min after exercise was lower after propranolol administration than without propranolol ($p<0\,01$) Also during exercise the propranolol pulse rate values were lower ($p<0\,001$)

The final exercise leukocyte count after propranolol administration averaged 7080 leukocytes /mm³ blood of which 58 9 % neutrophils and 32 9 % lymphocytes The corresponding resting values were 5990 59 0 and 33 8 The final exercise leukocyte count was higher than at rest ($p<0\,02$) though the numerical difference was small 1090 leukocytes/mm³ blood Also between the value after standing for 8 min—6000—and the final exercise value there was a statistical difference ($p<0\,05$)

After propranolol administration the leukocyte count—7080—at the first work load 300 kpm/

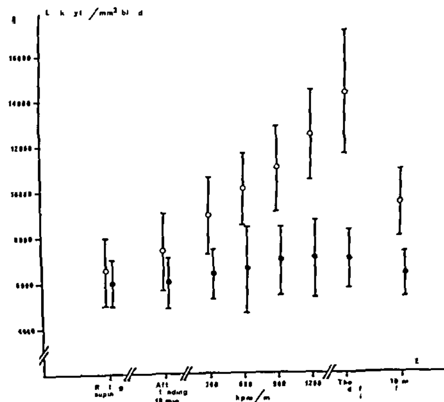


*Fig 1* Leukocyte m ans and 1 S.D in eight subjects before during and after the exercise test after propranolol administration (●) and without propranolol (○)

Table II *Pulse rates (beats/min) without (−) and with (+) propranolol during exercise in eight test subjects*

| Subject | Resting supine Day 1 − | Resting supine Day 2 − | Day 2 + | Standing − | Standing + | 300 kpm/min − | 300 kpm/min + | 600 kpm/min − | 600 kpm/min + | 900 kpm/min − | 900 kpm/min + | 1200 kpm/min − | 1200 kpm/min + | 1500 kpm/min − | 1500 kpm/min + | End of exercise − | End of exercise + | 10 min after exercise − | 10 min after exercise + | Duration of exercise (min) − | Duration of exercise (min) + | $W_{1\,0}$ (kpm/min) − | $W_{1\,0}$ (kpm/min) + |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 67 | 67 | 45 | 67 | 54 | 86 | 67 | 100 | 85 | 115 | 92 | 143 | 111 | 159 | 126 | 180 | 142 | 92 | 62 | 37 | 31 | 1700 | 2100 |
| 2 | 60 | 77 | 45 | 88 | 65 | 89 | 81 | 108 | 91 | 122 | 107 | 142 | 119 | 165 | 144 | 182 | 144 | 78 | 78 | 33 | 30 | 1550 | 1800 |
| 3 | 83 | 86 | 75 | 120 | 80 | 127 | 89 | 150 | 125 | 180 | 154 | 200 | 176 | | | 203 | 176 | 120 | 100 | 25 | 24 | 600 | 1150 |
| 4 | 67 | 76 | 65 | 92 | 67 | 101 | 88 | 123 | 107 | 132 | 122 | 175 | 140 | | | 188 | 161 | 102 | 82 | 28 | 27 | 1150 | 1650 |
| 5 | 72 | 75 | 61 | 89 | 74 | 110 | 93 | 142 | 116 | 172 | 138 | | | | | 187 | 152 | 92 | 72 | 21 | 20 | 900 | 1300 |
| 6 | 64 | 89 | 45 | 89 | 55 | 106 | 87 | 141 | 111 | 156 | 132 | 174 | 150 | | | 178 | 161 | 95 | 88 | 26 | 26 | 1150 | 1500 |
| 7 | 69 | 66 | 50 | 68 | 60 | 103 | 72 | 125 | 94 | 150 | 113 | 168 | 134 | 184 | 158 | 200 | 167 | 107 | 88 | 38 | 33 | 1200 | 1650 |
| 8 | 58 | 88 | 55 | 93 | 55 | 100 | 77 | 125 | 95 | 167 | 122 | 189 | 149 | | | 205 | 162 | 112 | 88 | 29 | 26 | 900 | 1400 |
| $\bar{x}$ | 67.8 | 78.0 | 55.1 | 88.3 | 63.8 | 102.8 | 81.8 | 127.0 | 103.0 | 151.8 | 122.5 | 170.1 | 139.9 | 169.3 | 142.7 | 190.4 | 158.1 | 99.8 | 82.3 | 29.6 | 27.1 | 1169 | 1569 |
| S.D. | 8.3 | 9.0 | 11.1 | 16.5 | 9.6 | 12.7 | 9.1 | 17.1 | 13.9 | 23.0 | 19.2 | 21.7 | 21.6 | 13.1 | 16.0 | 10.8 | 11.5 | 13.3 | 11.6 | 6.0 | 4.2 | 101 | 93 |
| Range | 58–85 | 66–89 | 45–75 | 67–120 | 54–80 | 86–127 | 67–93 | 100–150 | 85–125 | 115–180 | 92–154 | 142–200 | 111–176 | 159–184 | 126–158 | 178–205 | 142–176 | 78–120 | 62–100 | 21–38 | 20–33 | 800–1700 | 1150–2100 |

Table III *Leukocyte count (/mm³ blood) without (−) and with (+) propranolol during exercise in eight test subjects*

| Subject | Resting supine Day 1 − | Resting supine Day 2 − | Day 2 + | Standing − | Standing + | 300 kpm/min − | 300 kpm/min + | 600 kpm/min − | 600 kpm/min + | 900 kpm/min − | 900 kpm/min + | 1200 kpm/min − | 1200 kpm/min + | 1500 kpm/min − | 1500 kpm/min + | End of exercise − | End of exercise + | 10 min after exercise − | 10 min after exercise + |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 5300 | 4500 | 4900 | 5700 | 5200 | 7000 | 5000 | — | 3300 | 7900 | 5800 | 9000 | 6200 | 9300 | 7700 | 11500 | 6500 | 7500 | 6900 |
| 2 | 8600 | 6500 | 7100 | 9000 | 6000 | 11800 | 8300 | 12100 | 6200 | 12300 | 5900 | 12400 | 6500 | 13500 | 6800 | 16000 | 6800 | 8500 | 6300 |
| 3 | 6000 | 4900 | 5600 | 6500 | 6300 | 7500 | 6100 | — | 5800 | 10000 | 7100 | 11500 | 6000 | | | 11700 | 6000 | 9900 | 5600 |
| 4 | 5 00 | 6200 | 6400 | 6500 | 7400 | 8000 | 6300 | 8100 | 7300 | 11500 | 7300 | 12700 | 7400 | | | 13100 | 7500 | 11500 | 7500 |
| 5 | 5000 | 4000 | 4300 | 7900 | 4200 | 8100 | 5100 | 9900 | 4600 | 9900 | 6300 | — | | | | 12500 | 6600 | 8 00 | 4900 |
| 6 | 6100 | 4400 | 6000 | 6100 | 4900 | 9200 | 5900 | 9500 | 5500 | 11300 | 6000 | 12700 | 6000 | | | 15900 | 6000 | 9000 | 6000 |
| 7 | 6900 | 7200 | 7400 | 7000 | 6700 | 9400 | 7000 | 9500 | 10600 | 12200 | 10500 | 14300 | 10800 | 16500 | 7500 | 19300 | 9900 | 10900 | 7800 |
| 8 | 8700 | 6200 | 6200 | 10600 | 7300 | 11200 | 7200 | 12200 | 7100 | 14100 | 7200 | 15200 | 7100 | | | 15700 | 7300 | 11100 | 6200 |
| $\bar{x}$ | 6480 | 5490 | 5990 | 7310 | 6000 | 9030 | 6360 | 10220 | 6600 | 11140 | 7010 | 12570 | 7140 | 13100 | 7330 | 14460 | 7080 | 9580 | 6400 |
| S.D. | 1470 | 1180 | 1040 | 1670 | 1150 | 1730 | 1110 | 1620 | 1860 | 1870 | 1540 | 2020 | 1700 | 3620 | 470 | 2710 | 1 60 | 1490 | 970 |
| Rang | 5000–8700 | 4000–7200 | 4300–7400 | 5700–10600 | 4200–7400 | 7000–11800 | 5000–8300 | 8100–12200 | 4600–10600 | 7900–14100 | 5800–10500 | 9000–15 00 | 6000–10800 | 9300–16500 | 6800–7700 | 11300–19300 | 6000–9900 | 7500–11500 | 4900–7800 |

Table IV *Differential count in per cent of number of total leukoc*y*six of the test subjects without and with propranolol during exercise*

| | Day 1 | | | | | | Day 2 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ju venile neutro phils | Seg mented neutro phils | Baso phils | Eosino phils | Lym pho cytes | Mono cytes | Ju venile neutro-*phils* | Seg mented neutro-*phils* | Baso *phils* | Eosino *phils* | Lym pho cytes | Mono cytes | Ju venile neutro-*phils* | Seg mented neutro-*phils* | Baso-*phils* | Eosino *phils* | Lym pho-*cytes* | Mono *cytes* |
| | Without propranolol | | | | | | Without propranolol | | | | | | With propranolol | | | | | |
| *Resting* | | | | | | | | | | | | | | | | | | |
| $\bar{x}$ | 1 42 | 58 3 | 0 17 | 4 17 | 33 1 | 2 83 | 1 83 | 58 4 | 0 08 | 4 83 | 31 4 | 3 42 | 2 00 | 59 0 | 0 17 | 2 83 | 33 8 | 2 25 |
| S D | 1 07 | 3 7 | 0 26 | 1 81 | 6 1 | 1 29 | 1 57 | 3 4 | 0 10 | 3 92 | 3 8 | 2 04 | 2 22 | 5 5 | 0 26 | 2 58 | 4 2 | 1 13 |
| Range | 0 5–3 0 | 52 0–62 5 | 0 0–0 5 | 2 0–7 0 | 25 0–40 0 | 1 0–9 5 | 0 0–4 0 | 53 0–62 0 | 0 0–0 5 | 2 0–12 5 | 27 0–36 0 | 0 5–6 0 | 0 0–6 0 | 48 5–64 0 | 0 0–0 5 | 0 5–7 0 | 27 5–39 0 | 1 0–4 0 |
| | | | | | | | With propranolol | | | | | | | | | | | |
| *After 6 min exercise at 600 kpm/min* | | | | | | | | | | | | | | | | | | |
| $x$ | 1 67 | 61 3 | 0 33 | 4 17 | 29 7 | 2 83 | 1 42 | 60 3 | 0 0 | 3 83 | 32 2 | 2 33 | | | | | | |
| S D | 0 98 | 1 1 | 0 41 | 3 89 | 4 2 | 1 33 | 1 16 | 6 5 | 0 0 | 3 08 | 3 6 | 0 75 | | | | | | |
| Range | 1 0–3 5 | 60 0–63 0 | 1 0–1 0 | 1 0–11 5 | 22 0–34 0 | 2 0–5 5 | 0 0–2 5 | 49 5–68 0 | 0–0 | 0 5–9 0 | 26 0–36 0 | 1 0–3 0 | | | | | | |
| *At the end of exercise* | | | | | | | | | | | | | | | | | | |
| $\bar{x}$ | 1 25 | 67 6 | 0 0 | 4 25 | 25 2 | 1 75 | 1 25 | 58 9 | 0 0 | 3 92 | 32 9 | 3 00 | | | | | | |
| S D | 0 76 | 3 9 | 0 0 | 3 06 | 4 9 | 1 41 | 0 94 | 6 0 | 0 0 | 6 55 | 2 8 | 1 82 | | | | | | |
| Range | 0 0–2 0 | 62 0–72 0 | 0–0 | 1 0–10 0 | 19 0–32 0 | 0 0–3 0 | 0 5–3 0 | 48 5–65 5 | 0–0 | 0 5–17 0 | 29 0–37 5 | 0 0–4 5 | | | | | | |

min for 6 min did not differ from the final exercise mean value ($p>0.2$) that is after the initial rise during exercise there was no further rise of the leukocyte count during the last 21 2 min Without propranolol the rise during the corresponding period 23 6 min averaged 5430 leukocytes/mm³ blood ($p<0.001$)

Numerically the total neutrophil count without propranolol rose by 169% during exercise ($p<0.001$) After propranolol the corresponding rise was 17% ($p>0.2$) The total lymphocyte count rose by 74% during exercise without propranolol ($p<0.001$) and 16% after propranolol administration ($p<0.05$) As regards the resting values of lymphocytes neutrophils and leukocytes there was no difference between and after administration of propranolol

## DISCUSSION

During exercise the pulse rate increas was to a large extent limited by propranolol which has b en demonstrated by many authors (for review see 8) The increase in exercise leukocyte count was almost totally blocked by previous administration of propranolol Through the way in which the exp riments were planned and performed w tried to eliminate other influences on the exercise leukocytosis than propranolol administration The individual variations in leukocyte count from day to day might however have had some influence Still apart from this possibility it seems evident that exercise leukocytosis is mediated by a beta adrenergic receptor mechanism

The numerically small increas of exercise leukocytes after propranolol took place during the first minutes of exercise After the first load there was no further ris This initial increase might be explained by a decrease in blood volume because of exercise (6 13) and for orthostatic reasons It cannot be excluded either that the propranolol effect occurred only after some minutes of exercise The explanation as to why th re is still a slight rise in leukocyte count after the b ta receptor blockade lies in the fact that the beta adrenergic blockade cannot be complete at least with this dose of propranolol and probably not at all (4) This theory is also supported by the fact that the final heart rate was a little higher in our study than the intrinsic heart rate

Beta adrenergic blockade has been shown to reduce not only heart rate but also cardiac output and to increase the arteriovenous oxygen difference with subsequ nt reduction in the capacity for strenuous work (7) Our results as regards maximal work time are in analogy with these findings

As has been reported earlier the greater part of the increase in leukocyte count during exersice consists of segmented neutrophil leukocytes (2) A minor part consists of lymphocytes Concerning the neutrophil leukocytes calculated as percentage of the total leukocyte count from the differential count there was no statistically significant increase during work after propranolol For the lymphocytes there was a numerically small though significant increase Having regard to the small number of subjects however the cause is doubtful

## ACKNOWLEDGEMENT

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## REFERENCES


1 Ahlborg, B Capacity for prolonged exercise in man Forsvarsmed cin Suppl 1 1967
2 — Leukocytes in blood during prolonged physical exercise Forsvarsmedicin 3 36 1967
3 Ahlborg, B Magnusson, S & Zetterquist E In preparation
4 Chamberlain D A The haemodynamic effects of beta adrenergic blockade in man Symposium on propranolol Cardiologia (Basel) Suppl 2 27 1966
5 Cohen R. J Epstein S E Cohen L S & Dennis L H The role of exercise and β adrenergic stimulation on blood coagulation and fibrinolysis Circulation Suppl. 6 57 1968
6 Ekelund, L-G & Holmgren A Circulatory and respiratory adaptation during long term non steady state exercise in the sitting position Acta physiol scand 6? 240 1964
7 Epstein S E Robinson B F Kohler R L & Braunwald E Effects of beta adrenergic blockade on the cardiac response to maximal and submaximal exercise in man J clin Invest 44 1745 1965
8 Furberg, C Effects of beta adrenergic blockade on ECG phys cal working capacity and central circulation with spec al reference to autonomic imbalance Acta med scand. Suppl. 488 1968
9 Holmgren, A. & Mattsson, K. H A new ergometer with constant work load at varying pedalling rate Scand. J clin Lab Invest. 6 137 1954
10 Johnsson, G Norrby A. & Solvell L. Potency and time-effect relationship in man of propranolol and H 56/28 Acta pharmacol (Kbh.) Suppl. 2 95 1967

11 Schultz, G Experimentelle Untersuchungen uber das Vorkommen und die diagnostische Bedeutung der Leukocytose Dtsch. Arch. klin. Med. *51* 234 *1893*
12 Sjostrand, T Functional capacity and exercise tolerance in patients with impaired cardio-vascular function Clinical and cardiopulmonary physiology Grune & Stratton New York *1960*
13 Uehnger A. & Buhlman A Das Verhalten des Blutvolumens wahrend kurzfristiger korperlicher Arbeit Cardiologia (Basel) *38* 357 *1961*

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# ORAL L-DOPA TREATMENT OF PARKINSONISM

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*Abstract* Six parkinsonian patients have been treated with increasing oral doses of *L*-3 4-dihydroxyphenylalanine (*L*-dopa) up to 8 25 g/d. Previous anticholinergic therapy had been discontinued. Four of the patients were remarkably improved the best effect was observed on the akinesia. The *L*-dopa treatment was discontinued in two of the patients as they were not markedly improved but displayed considerable side-effects. Nausea and occasionally vomiting were observed in most patients. Hyperkinesia occurred at the high dose levels. Their mood was elevated but mental disturbance developed in four of the patients. In two of these patients the disturbance disappeared after reduction of the dose Most of the *L*-dopa was metabolized to dopamine outside the brain. Large diurnal variations of the dopa level in blood were observed in agreement with similar variations in the therapeutic effect.

About ten years ago it was found that dopamine (DA) occurs in the mammalian brain and that most of this amine is present in the neostriatum (the caudate nucleus and putamen) and the substantia nigra It was also found that reserpine depletes these DA stores and that *L*-3 4-dihydroxy phenylalanine (*L*-dopa) restores both the level of DA in the brain and the behavior of the reserpine treated animals (7) Soon afterwards Hornykiewicz (13) showed a very marked reduction of DA in the neostriatum and in the substantia nigra of patients with parkinsonism The pathophysiology of human parkinsonism may be at least partly explained by an impairment of the nigro-neostriatal DA neuron system described by Anden et al (1) and Bertler et al (6) Since the degeneration of these neurons is probably not complete and since the dopa decarboxylation is not rate limiting the lack of DA might be counteracted by administration of *L*-dopa In support of this hypothesis parenteral treatment of parkinsonism with this compound has been reported to partially relieve akinesia However clinically useful results could generally not be obtained largely due to side-effects (13) Similarly oral treatment with small doses of dopa was only partially successful (4) Recently Cotzias et al have reported considerably more successful results by chronic oral treatment of parkinsonism with large doses of *DL* (10) or *L*-dopa (9) These studies initiated the present investigation in which the response of parkinsonian patients to orally administered *L*-dopa has been followed by means of clinical observations tests of physical ability and mechanographic recordings In addition some data on the metabolism of *L*-dopa in these patients are given

## MATERIAL AND METHODS

The present investigation was performed on six parkinsonian patients who were all severely incapacitated. Some of the clinical data are given in Table I. Four of the patients had been hospitalized for at least one year All the patients were hospitalized for at least six months from the start of the study The parkinsonian symptoms present in each patient at the start of the study can be seen in Table II which gives a summary of the effect of *L*-dopa treatment on these symptoms. Prior to the study *L*-dopa was described to them as a new drug, which according to previous experience might be of value for some but not all patients with Parkinson s disease They were also informed that the treatment might involve risks. All the patients were considered capable of understanding this information and declared that they were willing to participate in the study

### *Drug Therapy*

The previous anticholinergic therapy was discontinued and no antiparkinsonian drugs were given for two weeks except in case 5 in whom some anticholinergic medication was necessary in the initial stage due to bronchial hyper

Table I *Case material*

| Case | Sex | Born | Start of disease | Stereotaxic operation | Previous anti parkinsonian medication | |
|---|---|---|---|---|---|---|
| 1 | o | 1920 | 1955 | 1959 Thalamotomy bilat | Trihexyphenidyl<br>Orphenadrine | 5 mg × 1<br>50 mg × 1 |
| 2 | ♂ | 1893 | 1956 | — | Trihexyphenidyl | 5 mg × 3 |
| 3 | ♂ | 1910 | 1963 | — | Orphenadrine | 50 mg × 4 |
| 4 | ♂ | 1910 | 1957 | 1965 Thalamotomy *left* side | Trihexyphenidyl<br>Orphenadrine<br>Benztropine | 2 mg × 3<br>50 mg × 3<br>2 mg × 3 |
| 5 | ♂ | 1906 | 1957 | — | Trihexyphenidyl<br>Orphenadrine<br>Benztropine | 5 mg × 3<br>50 mg × 3<br>2 mg × 2 |
| 6 | ♀ | 1896 | 1959 | — | Orphenadrine | 50 mg × 3 |

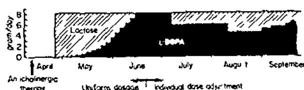



*Fig 1* Schedule of *L*-dopa administration

secretion At the end of this period the basal state of the patients was registered. The treatment was then begun with a two week period of lactose administration Succes vely increasing doses of *L*-dopa were then given The ve drug (*L*-dopa) and the placebo (lactose) were tered in capsules of identical size and colour (*L* from Ajinomoto Co Tokyo Japan before use the substance was analysed both by thin layer chromatography (ascending, *n* butanol pyridine $H_2O$ = 1 1 1) and by spectrofluorimetry and found not to contain any significant impurity) Initially the capsules were administered three times daily (in general at 8 a m 1 p m. and 6 p m) Later up to five daily doses have been given. The active capsules contained 100 200 and 250 mg respectively of *L*-dopa in order to enable a continuous increase and a fine adjustment of the dosage The schedule of administration of the drug (Fig. 1) was in general identical to that described by Cotzias (9) The maximum dose used was 8 25 g per day The patients were always given the same number of capsules per day and were never told that some capsules contained an inactive sub tance After the maximum dose was reached it was slowly reduced until the parkinsonian symptoms reappeared or became more severe The dose for each patient was then adjusted in order to reach an optimum dose level regarding both therapeutic response and side-effects

## *Physical Therapy*

All patients had received physical therapy aimed at counteracting rigidity muscular atrophy and invalidity continuously or periodically for several years. During the present study the same training programme was used whether placebo or *L*-dopa was given Naturally this training programme could be more effectively fulfilled when the patient improved due to the *L*-dopa administration

## *Functional Tests*

A careful general clinical examination and the following recordings were made to evaluate the effect of the *L*-dopa treatment

1 Handwriting.

2 Ability to draw with each hand a triangle a square a circle and a spiral

3 Number of steps when walking 10 m

4 Time needed for walking 10 m.

5 Time needed to rise from and return to sitting position

6 Time needed to put on a pair of socks The test reflects inter alia a disability of hand motility

7 Colour cinematography of movements according to a standardized schedule

8 Nursing load points This method evaluates the patient s ability to take his clothes on and off to eat wash walk defecate and urinate himself It further records any disturbance which the patients may cause and the possible occurrence of bedsores The maximum number of points a patient can receive using this method of evaluation is 41 (14)

9 Mobility points. Zero to 9 points represent different degrees of motor impairment in a patient able to walk 10 to 19 in a wheel-chair patient 0 to 30 in a patient confined to bed (11)

10 Mechanographic examination The method was introduced for measuring hypokinesia (16) Governed by a tape recorder program a spot of light was moved horizontally on a screen in front of the patient The sitting patient handled a second spot of light with a lever in his right hand and was instructed to follow the first moving spot as closely as possible The movements were recorded on channels 1 and 2 of a four-channel Grass polygraph. The difference between the two records, the error signal, was fed to the third channel The fourth channel demonstrated the integrated error Triangular sinusoidal rectangular and irregular movement programmes were presented during a period of five minutes. The akinesia was

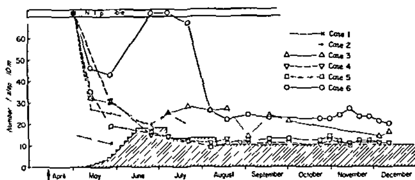


*Fig* 2 Number of steps required for walking 10 m.

manifested in a phase lag and low amplitude of the movements Total akinesia, i e when the lever was not moved at all was regarded as 100 % error Inability to follow the spot closely introduced the phase lag factor thus enabling the error factor to rise above 100 % Other factors which might contribute to the error apart from hypokinesia, were tiredness, distraction and hyperkinesia but these could usually be distinguished by the form of the curves The patient was continually observed during the test Healthy young individuals showed about 25 % error

### *Laboratory investigations*

11 The following clinical or laboratory examinations were performed before treatment and at different stages throughout the study in order to detect side-effects on the thyroid gland and on the haematological, cardiovascular hepatic, renal and gastrointestinal system. body weight, rectal temperature heart rate systemic blood pressure (recumbent and upright) electrocardiography X ray of heart and lungs, ophthalmological examination, electroencephalography haemoglobin, red blood count, white blood count, differential blood count, blood platelet count, erythrocyte sedimentation rate thymol turbidity serum bilirubin, serum alkaline phosphatase serum glutamic oxalacetic transaminase serum glutamic pyruvic transaminase serum creatinine protein bound iodine blood glucose serum electrolytes (sodium potassium calcium, chloride bicarbonate phosphate protein) urine analysis (pH protein glucose ketones blood) microscopical examination of urinary sediment and Weber's test for occult faecal blood

12 In order to study the metabolism of *L*-dopa, samples from plasma or 24 h urine were examined for dopa (5) 3 methoxytyrosine (3-O methyl-dopa) (12) and DA (8) spectrofluorimetrically after cation exchange chromatography and oxidation Homovanillic acid (HVA) in the cerebrospinal fluid (CSF) plasma and urine was determined spectrofluorimetrically after organic solvent extraction and oxidation (2) In addition, 5 hydroxyindole acetic acid (5 HIAA) in the CSF was determined spectrofluorimetrically (3)

## RESULTS

### *Effect on Parkinsonian Symptoms*

The recordings of data started shortly after the discontinuation of the anticholinergic therapy (Figs 2–7) The parkinsonian symptoms became markedly worse in at least five of the six patients during the period without antiparkinsonian drugs During the two weeks of placebo treatment the symptoms were unchanged or in some patients even more severe

In four of the patients (cases 3–6) remarkable improvement was observed during the *L*-dopa treatment In case 2 some improvement was recorded after *L*-dopa treatment This improvement was not obviously better than that obtained after

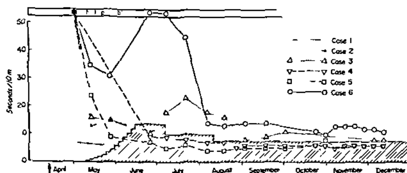


*Fig* 3 Time in seconds required for walking 10 m.

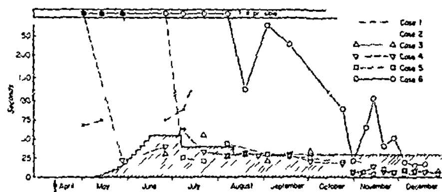



*Fig 4* Time needed to put on a pair of socks.

anticholinergic therapy and due to some undesirable side-effects (see below) of *L*-dopa the treatment was discontinued In case 1 a serious condition developed which may or may not be ascribed to the treatment In this case the *L*-dopa treatment was also discontinued A summary of therapeutic responses side-effects and complications is shown in Tables II and III The time course of the improvement was variable as shown in Figs 2–7 A certain improvement was observed in a few patients already during the first few weeks of treatment In general a striking response as not seen until several weeks after the onset of tment and when a dose level of several grams per day had been reached In two patients (cases 4 and 6) even after an optimum dose level was reached their condition varied markedly during the day The symptoms were rather severe in the morning and disappeared more or less completely about two hours after the first morning dose of the drug Similar variations were seen in other patients Of the three major symptoms of parkinsonism the most pronounced and earliest effect was on akinesia The least pronounced effect though clear-cut in three of the patients was on the tremor Case 4 had undergone a stereotaxic operation on the left side in 1959 which had a good effect on the tremor on the right side While

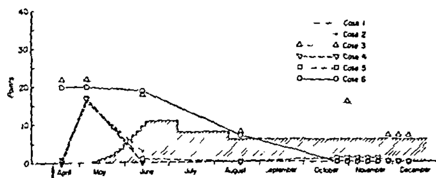



*Fig 5* Nursing load points.




*Fig 6* Mobility points.

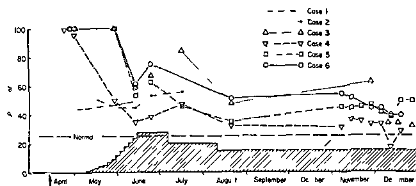



*Fig 7* Mechanography

on *L*-dopa treatment the akinesia improved more in the left than in the right arm

Three of these patients (cases 4–6) left the hospital about five months after the *L*-dopa treatment began and have since been followed by weekly examinations Some minor adjustments in dosage have been made After eight months treatment the doses are case 6 3 0 g/d case 5 4 8 g/d case 3 5 4 g/d case 4 6 0 g/d Mental depression which occurred in at least two of the patients disappeared during *L*-dopa treatment Also in the other patients the mood appeared to be elevated during this treatment

Table II *Effect of L dopa treatment on parkinsonian symptoms and disabilities*

| Case | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Hypokinesia (Fig 7) | NP | — | — | — | — | — |
| Rigidity | 0 | 0 | — | — | — | — |
| Tremor | 0 | 0 | NP | — | — | — |
| Flexion posture | NP | 0 | — | — | NP | — |
| Gait disturbance (Figs 2 and 3) | NP | — | — | — | — | — |
| Mobility defect (Fig 6) | NP | — | — | — | — | — |
| Hypersalivation | NP | — | — | NP | — | NP |
| Speech disturbance | 0 | — | — | NP | 0 | — |
| Hypomimia | 0 | — | — | — | 0 | — |
| Writing and drawing difficulty | 0 | — | — | NP | — | — |
| Difficulty in dressing (Fig 4) | 0 | — | — | — | — | — |
| Need of care (Fig 5) | NP | — | — | — | — | — |
| Mental depression | NP | NP | — | NP | NP | — |

Pathological manifestations reduced or abolished during treatment —
Pathological manifestations not influenced during treatment 0
Pathological manifestations not present before the start of the treatment NP

*Side-effects and Complications* (Table III)

Nausea and occasionally vomiting were observed in most patients especially after increasing the dose of *L*-dopa It usually disappeared after a few days even without a reduction in the dose These side effects never necessitated discontinuation of the treatment A certain degree of nausea was preferred to the parkinsonian symptoms by the patients No significant change in body weight occurred during *L*-dopa treatment

Hyperkinesia was observed in three patients but disappeared when the dose was lowered The hyperkinesia was manifested as involuntary movements of face shoulder and leg muscles

Retraction of the eye lid was observed in most patients while on *L*-dopa treatment Acute transient conjunctivitis was observed in four patients

In two of the patients orthopedic complications fracture of the femoral neck (case 2) and luxation of the shoulder (case 4) occurred A causal role of *L*-dopa is dubious but cannot be excluded in view of the increased motility and the possible loss of judgement.

Mental disturbances were observed in four pa

Table III *Side effects and complications appearing during L-dopa treatment*

| Case | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Nausea and vomiting | (+) | (+) | (+) | (+) | | + |
| Hyperkinesia | | | (+) | + | | (+) |
| Eye lid retraction | + | + | + | | + | |
| Conjunctivitis | + | + | + | | + | |
| Dizziness | + | + | + | (+) | | (+) |
| Orthopedic complications | | + | | + | | |
| Mental disturbances | + | + | | | + | (+) |

Pathological manifestations induced during treatment +
Only occasionally (+)

Table IV *Urinary excretion (mg per 24 h) of dopa and some of its metabolites and CSF levels (μg/ml) of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) before and during treatment with L-dopa*

| | | Urine | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Daily dose of *L*-dopa | Dopa | | 3-methoxy tyrosine | | Dopamine | | | CSF | |
| Case | (g) | Conjugated | Free | Conjugated | Free | Conjugated | Free | HVA | HVA | 5-HIAA |
| 1 | 0 | ND | 0.1 | ND | 0.0 | ND | 0.1 | ND | 0.01 | 0.0[illegible] |
| 2 | | ND | 0.1 | ND | 0.0 | ND | 0.2 | 0.9 | 0.01 | 0.02 |
| 3 | | ND | 0.0 | ND | 0.2 | ND | 0.0 | ND | ND | 0.02 |
| 4 | | ND | ND | ND | ND | ND | ND | ND | 0.01 | 0.03 |
| 5 | | ND | 0.0 | ND | 0.0 | ND | 0.1 | 0.4 | 0.00 | 0.05 |
| 6 | | ND | ND | ND | ND | ND | ND | ND | 0.02 | 0.02 |
| 1 | 1.2 | ND | 1.4 | ND | 8 | ND | 26 | [illegible] | ND | ND |
| 2 | | ND | 0.8 | ND | 4 | ND | 25 | 300 | ND | ND |
| 3 | | ND | 0.8 | ND | 6 | ND | 32 | 340 | ND | ND |
| 4 | | ND | 3.0 | ND | 32 | ND | 41 | 330 | ND | ND |
| 6 | | ND | 0.5 | ND | 2 | ND | 13 | 43 | ND | ND |
| 2 | 8 | ND | 51 | ND | 140 | ND | 480 | 1160 | 0.38 | 0.04 |
| 3 | | ND | 116 | ND | [illegible] | ND | 410 | 580 | 0.48 | 0.02 |
| 4 | | ND | 188 | ND | 530 | ND | 560 | [illegible] | 0.73 | 0.02 |
| 5 | | –1 | 29 | 14 | 80 | 320 | 140 | 990 | 0.65 | 0.04 |
| 6 | | ND | 177 | ND | [illegible] | ND | 550 | 810 | 0.77 | 0.02 |
| 2 | 6* | 21 | 71 | 69 | 123 | 530 | 400 | 1330 | 0.50 | ND |
| 3 | | 73 | 85 | 42 | 136 | 950 | 540 | 12 0 | 1.17 | 0.02 |
| 4 | | ND | 50 | ND | 150 | ND | 290 | 810 | 0.21 | 0.02 |
| 5 | | 1 | 19 | 24 | [illegible] | 105 | [illegible] | 1410 | 0.62 | 0.03 |
| 6 | 3* | 3 | 9 | 0 | 32 | 260 | 130 | [illegible] | 0.34 | 0.01 |

* Optimum dose
ND – not determined.

Table V *Plasma levels (μg/ml) of dopa 3 methoxytyrosine and homovanillic acid (HVA) at different times during the day in case 6*

The *L*-dopa was given orally in three doses daily each of *1 g at 8 a.m. 1 p.m. and 6 p.m*

| Time | Dopa | Methoxy tyrosine | HVA |
|---|---|---|---|
| 8 a.m. | 0.02 | 0.59 | 0.17 |
| 10 a.m. | 1.92 | 2.01 | ND |
| 2 p.m. | 1.19 | 1.32 | 1.85* |
| 5 p.m. | 0.89 | 1.37 | 1.25 |

* Simultaneous CSF value 0.34 μg/ml.
ND – not determined.

tients. Case 6 displayed somnolence and disorientation while on a dose of 6 g/d *L*-dopa was then discontinued and the symptoms disappeared. After a few days the *L*-dopa treatment was reinstated and the dose was increased at a much slower rate without any mental disturbances. Case 5 whose parkinsonian symptoms largely disappeared after a dose of 4.8 g/d. apart from amimia and severe aphonia, responded to an increase up to 6.0 g/d. with overactivity (apparently compulsive hammering, painting, etc.) even at nights. After reduction of the dose to 4.8 g/d these symptoms disappeared. Case 2 developed hallucinations and hypomania during the *L*-dopa treatment but these symptoms disappeared when the dose was lowered. In one patient (case 1) more serious mental changes developed during the study. His first symptom of Parkinson's disease had appeared in 1955. Anticholinergic therapy was tried but elicited states of confusion. In 1959 a stereotaxic thalamotomy was performed on the left side and later in the same year on the right side. On these occasions pneumoencephalography demonstrated enlargements of the cerebral ventricles. The parkinsonian symptoms were considerably improved but deficiency of speech and mouth movement control appeared. The patient had displayed a

slight but progressive intellectual impairment during the last years Successively the parkinsonian symptoms reappeared and at the start of the present study his main physical impairment was a tremor of the left hand and to a lesser extent of the right hand and left leg He had moderate rigidity and akinesia. No or a very slight improvement of the objective tests was noted during the period of increasing *L*-dopa administration (Figs 2–7) Some days after the maximum dose was reached he displayed some confusion, which was rather rapidly aggravated The *L*-dopa administration was then discontinued During the next days the patient developed olfactory hallucinations incomprehensible speech and urinary incontinence The confusion successively subsided but never completely Six weeks after the discontinuation of the *L*-dopa treatment he had an epileptic seizure EEG demonstrated no focal abnormalities Pneumoencephalography showed general cerebral atrophy After this epileptic attack the patient rapidly deteriorated and was in a condition of stupor for several weeks Some improvement has since occurred The patient is now conscious but confined to bed.

Apart from the above mentioned side-effects no complications were revealed in the examinations mentioned above In particular there were no abnormalities in the total or differential leucocyte count, no hypertension or orthostatic phenomena no ECG changes and no influence on the motility of the gastrointestinal tract or urinary bladder

### *Monoamine Metabolism*

*Urine* (Table IV)

During the treatment with *L*-dopa significant amounts of this compound were observed in the urine At the higher doses 0 3–1 4% of the administered dose was found in the urine Acid hydrolysis of the urine did not give any significant increase in the amount detected The excretion of 3 methoxytyrosine was of about the same magnitude but usually somewhat higher A large part of the DA excreted was conjugated The total DA excreted at the higher dose levels corresponded to 4–25 of the dose of *L*-dopa given. The percentage excretion of free DA appeared to be higher at high rather than at low dose levels One of the major metabolites of *L*-dopa in the urine was HVA which varied between 12 and 28% of the dose given At the dose level of 3–6 g *L*-dopa, the dopa and the observed metabolites in the urine amounted to 20–52 of the dose of *L*-dopa given

*Blood* (Table V)

The plasma was analysed in one of the patients (case 6) when she received three doses of 1 g *L*-dopa each during the day Before the first daily dose no dopa could be detected in the plasma Significant levels of dopa were obtained 1–4 hours after the administration. 3 methoxytyrosine and HVA in plasma showed similar variations The levels of each of these three compounds were of the order of 1–2 $\mu$g/ml

*CSF* (Table IV)

The level of HVA was between 0 00–0 02 $\mu$g/ml in all patients before the treatment with *L*-dopa started, as compared to a normal range of 0 01–0 13 $\mu$g/ml (15) At the optimum doses the HVA level was increased to 0 2–1 2 $\mu$g/ml The levels of 5 HIAA before treatment were between 0 02–0 05 $\mu$g/ml (normal range 0 02–0 06 $\mu$g/ml (15)) No significant change occurred during the treatment with *L*-dopa

## DISCUSSION

As mentioned above the present study was initiated after the remarkably successful results of *L-dopa treatment in parkinsonism were reported by* Cotzias et al (9 10) It should be considered as a pilot study with the following main purposes

(*a*) To study the effect of *L*-dopa by means of objective clinical tests

(*b*) To study the value of mechanographic and biochemical investigations in selecting patients suitable for *L*-dopa treatment.

(*c*) To study the changes in monoamine metabolism during the treatment and to relate them to the clinical effects

The results of this pilot study have encouraged us to continue and extend the investigation *L*-dopa treatment has been started in 14 additional parkinsonian patients and the biochemical investigation has been further extended.

Concerning the first purpose the results of the present investigation agree on the whole with the

observations of Cotzias et al (9 10) After the initiation of our study a paper by Yahr et al (17) appeared likewise confirming the results of Cotzias et al Like the previous workers we observed the strongest effect on akinesia and the weakest on tremor

In the patients reported on in the present paper we stopped anticholinergic therapy four weeks before starting *L*-dopa treatment, since like Cotzias et al. we wanted to study the effect of *L*-dopa treatment alone We observed a marked deterioration of the condition in some of the patients indicating that anticholinergic therapy was effective Since the period between interruption of anticholinergic therapy and institution of *L*-dopa was only four weeks our data do not demonstrate a stable baseline level before starting *L*-dopa treatment However in our subsequent investigation we have kept the patients on anticholinergic treatment throughout the investigation. The sequence of events on this combined regimen of anticholinergics and *L*-dopa is similar to that observed in the present study

In those four patients described in the present paper in whom a marked improvement following treatment was observed *L*-dopa appeared clearly superior to anticholinergic therapy Ac to our present preliminary impression based on observations made later on combination of *L*-dopa with anticholinergic therapy appears to be superior to either drug alone at least in certain patients.

Concerning the problem as to which patients should be selected for *L*-dopa treatment, the present pilot study seems to indicate that the treatment should be given preferably to patients with marked akinesia and rigidity This conclusion is derived from the results of the mechanographic investigation which showed that, in the two patients (cases 1 and 2) in whom the akinesia was rather slight, the effect of *L*-dopa was also slight or absent. This view on the preferable selection of patients for *L*-dopa treatment is of course also supported by the clinical impression that the strongest effect was on the akinesia.

In this preliminary material none of the biochemical investigations performed prior to the *L*-dopa administration (Table IV) showed any variations that could b related to the clinical effect of the drug.

Concerning the third purpose mentioned above *L*-dopa administration was accompanied by a considerable increase in urinary excretion of dopa and its metabolites as might be expected A marked increase in the level of HVA in CSF was also observed In these respects no conspicuous difference was observed between patients responding favourably and those in whom little or no favourable response was observed The few observations made on dopa in plasma suggest that a correlation between plasma level and clinical effect may exist We therefore intend to investigate this problem further

Hitherto no serious side-effects of *L*-dopa have been described in the literature It is difficult to decide whether the marked deterioration observed in case 1 was the result of the *L*-dopa treatment and if so what role the previous bilateral thalamotomy and cerebral atrophy may have played Cotzias et al and Yahr et al as well as we ourselves have treated a number of other thalamotomized patients with favourable results Whether these patients in addition suffered from cerebral atrophy is not known Anyhow we judged it important to describe the present case in detail in view of the relatively few reports of *L*-dopa treatment in the literature

## ACKNOWLEDGEMENTS

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## REFERENCES

1 Anden N E Carlsson, A, Dahlstrom, A Fuxe K, Hillarp N A & Larsson, K. Life Sci 3 523 1964
2 Anden, N E Roos, B E & Werdinius B Life Sci. 2 448 1963
3 Ashcroft, G W & Sharman, D F Brit J Pharmacol 19 153 1962.
4 Barbeau, A Canad. med. Ass. J 87 80., 196.
5 Bertler A Carlsson, A & Rosengren, E Acta physiol. scand. 44 273 1958
6 Bertler A., Falck, B., Gottfries, C-G Ljunggren, L & Rosengren, E. Acta pharmacol. (Kbh.) 21 283 1964
7 Carlsson, A In. Biochemistry and pharmacology of the basal ganglia. Proceedings of the Second Symposium of the Parkinson's Disease Information and Research Center (ed E Costa, L J Coté & M D Yahr) p 107 Raven Press, Hewlett, New York 1966

8 Carlsson, A. & Waldeck, B Acta physiol. scand 44 293 1958
9 Cotzias G C Papavasiliou, P S & Gellene R. New Engl J Med. 280 337 1969
10 Cotzias, G C Van Woert, M H & Schiffer L. M New Engl. J Med. 276 374 1967
11 Gillner A., Hultén, A. Kerstell, J Olsson R. Steen B & Svanborg A Scand J rehab Med. In press
12. Gjessing L. E. Vellan, E. J Werdinius B & Corrodi H Acta chem. scand 21 8.0 1967
13 Hornykiewicz, O Pharmacol Rev 18 925 1966
14 Hultén, A., Kerstell J Olsson, R. & Svanborg, A. Scand. J rehab Med. In press.
15 Johansson B & Roos, B E. Life Sci 6 1449 1967
16 Johnels, B & Steg, G To be published.
17 Yahr M D Duvoisin R. C Hoehn M M Schear M J & Barrett R. E Trans Amer neurol Ass 93 56 1968

## ADDENDUM

After this paper was submitted (July 1969) the number of patients treated by us with *L*-dopa has increased to 47 the observation time being up to 19 months (December 1969) This extended material confirms in general our earlier observations however in three male patients a strong aphrodisiac effect of the drug was observed Furthermore the effect of *L*-dopa on tremor has some times been found to improve markedly during long term treatment.

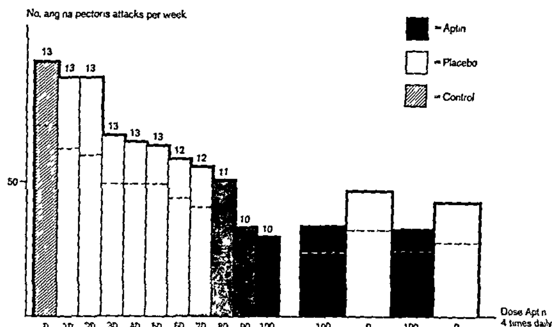
During the publication time several other reports (1 2 3 4) on *L*-dopa treatment have appeared, largely in agreement with our observations.

*References*

1 Calne D B Stern G M & Laurence D R. Lancet 7598 744 1969
2 Calne D B Spiers, A S D Stern G M., Laurence D R. & Armitage P Lancet 7628 973 1969
3 Godwin Austen R. B Tomlinson E B., Frears, C C & Kok, H W L Lancet 7613 165 1969
4 Yahr M D Duvoisin R. C Schear M J Barrett, R. E & Hoehn M M Arch Neurol (Chic) 21 343 1969

# Double blind study of the action of APTIN® (alprenolol) and placebo in angina pectoris

Bjorntorp, P Acta Med Scand 182 285, 1967




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Acta med scand Vol 187 pp 257–265 1970

# OBSERVATIONS REGARDING THE NATURE OF HOWELL JOLLY BODIES

Nils Soderstrom and Bertel Berg

*From the Med cal Department A Un ersity Hospital Lund Sweden*

*Abst act* A study of the distribution and morphology of Howell Jolly bodies in blood, bone marrow and spleen aspirates from patients with and without functionin spleen led to the conclusion that most bodies so labelled are not intracellular remnants of the normoblast nuc ei. They are in most cases situated on the outside of the erythrocyte membrane and to be regarded as degradation products of nuclei digested by bone marrow phagocytes and released from these cells at a certain stage of de gradation. They are normally removed by the spleen in the absence of the spleen they may remain for some time in circulating blood with a tendency to adhere to red cells.

Since the beginning of this century textbooks on hematology traditionally mention Howell Jolly (HJ) bodies together with Cabot s ring as residues of the normoblast nucleus in red cells Nowadays it is usually added that this interpretation of Ca bot s rings is doubtful HJ bodies still hold their place as nuclear remnants

They do it by virtue of the fact that they contain DNA and it is hard to imagine anything else than the normoblast nucleus as the source of DNA within red cells Convincing arguments for this were given already by Howell (6) and Jolly (7 8) and more evidence has been added later on e g. Feulgen positivity (see Bessis (1))

Howell observed these bodies accidentally in blood from *normal* kittens in the regeneration phase after severe blood loss but HJ bodies be came something more than a mere curiosity with the observation by Schur (10) that they turn up in blood after *splenectomy* It is now generally ac cepted that in man and many animals the absence of a functioning spleen is almost constantly sig nalled by the presence of HJ bodies in blood no matter whether this asplenia is congenital or ac quired (5) In different blood disorders they may be found also in the presence of a presumably functioning spleen (see de Gruchy (4)) but a de finition of HJ bodies has always to consider this specific relation to asp enia

The authors venture to define HJ bodies as sharply demarcated rounded bodies with the staining properties of nuclear DNA which in blood smears are found usually singly within the area of red cells The typical HJ body has a diameter of about 1 $\mu$

This question of size has some important impli cations The range of variation has a very sharp *upper* limit HJ bodies larger than 1.5 $\mu$ across are hardly ever se n This is remarkable in view of the fact that the minimum diameter of normo- blast nuclei is about 3 5 $\mu$ nuclear bodies of transitional dimensions to bridge this hiatus are only exceptionally seen in red cells

On the other hand there is no clear-cut lower size limit to distinguish HJ bodies from different types of smaller bodies stainable with nuclear stains and often barely visible with the light microscope One type of such small bodies were found by Reimann (9) to stick to the *outside* of red cells Also these bodies se med to increase in numbers after splenectomy but Reimann regarded them as fundamentally different from HJ bodies In any case it is probably wise to reserve the label HJ body for larger specimens with a diameter *well* above 0 5 $\mu$ This is an artificial size limit but it helps provisionally to define the conspicu ous bodies which are well known by every hema tologist as typical findings in blood after splenec tomy

The accepted views on the nature of HJ bodies contain some obscure points It may be imagined that the spleen has to pick up HJ bodies or cells containing HJ bodies during the passage of blood through its parenchyma, but how is this difficult

task accomplished? Some authors even postulate that the spleen acts by some distant influence on the denucleation of red cells in the bone marrow It might thus be of interest to investigate whether HJ bodies are normally present in the bone marrow and whether their formation mechanism could be elucidated by morphologic studies in erythropoietic cells

Bone marrow studies might especially be expected to solve the problem of the curious "hiatus" in size between normoblast nuclei and HJ bodies (volume relations in the order of 50/1[1]) If HJ bodies are really remnants of the normoblast nucleus nuclear bodies of transitional dimensions should be present in the bone marrow

The main topic of the present paper will thus be a study of HJ bodies in human bone marrow supplemented by some observations regarding HJ bodies in spleen aspirates and in peripheral blood in conditions of asplenia

## MATERIAL AND METHODS

The starting point of the present study was a case of nontropical sprue in which no spleen could be recognized in a celiac arteriography made for other purposes. The suspicion of splenic atrophia could immediately be supported by the detection of numerous HJ bodies in a blood smear (1 400 cells) They proved to be still more plentiful in the bone marrow thus made us extend the study to the following material

1 Blood and in one case bone marrow aspirate from seven patients without blood disorders, who had earlier been *splenectomized* after traumatic rupture of the spleen

2 Blood, bone marrow aspirate and in five cases preoperative spleen aspirate from 11 patients *splenectomized* for different blood disorders, five cases of idiopathic thrombocytopenic purpura, three cases of hemolytic anemia, one case of chronic lymphatic anemia with thrombocytopenia and two cases classified as splenic lymphosarcoma. In four of these patients the first appearance of HJ bodies was looked for in serial blood smears 4-36 hours after the operation

3 Blood, bone marrow aspirate and in five cases spleen aspirate from 40 consecutive *non splenectomized* patients subjected to bone marrow aspiration because of suspected or overt blood disorders

The standard material scrutinized for HJ bodies was air-dried smears stained with May-Grunwald-Giemsa The following additional methods were applied in some of the cases

A. Air-dried smears, staining with methyl green, trypan blue, bromphenol blue, Feulgen reaction Prussian blue reaction.

Treatment with DNAse (DNAse 1 from bovine pancreas, Sigma).

B. Wet preparations, direct study of native blood in cover glass preparations the staining reaction with trypan blue and neutral red in isotonic and hyposmotic *milieu* reticulocyte staining with brilliant cresyl blue according to Bjorkman.

C Clotted peripheral blood was scrutinized for HJ bodies in 1 $\mu$ sections by phase contrast light microscopy and in ultrathin sections using low power electron microscopy (glutar aldehyde epon, osmium tetroxide lead citrate)

## RESULTS

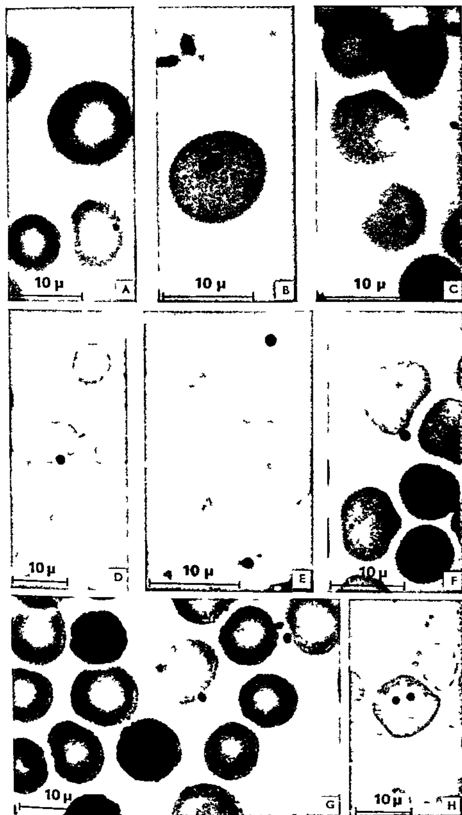
### (a) HJ bodies in peripheral blood

In blood smears from the 40 *non splenectomized* patients a few HJ bodies were seen in four cases representing two cases of megaloblastic anemia one case of chronic myeloid leukemia and one case of apparently primary hypoplastic anemia HJ bodies in these cases were too scarce to permit special studies but it should be mentioned that in *this* material there were several examples of 2-3 HJ bodies in the same cell (Fig. 1 H)

In all the 18 *splenectomized* cases typical HJ bodies were found in smears of capillary blood with an incidence ranging between a few bodies scattered over the smear and a maximum yield of 1 HJ body/130 red cells in a patient with hemolytic anemia splenectomized two months before the examination. Only exceptionally was more than one HJ body seen in each cell in the maximum yield case mentioned above two separate HJ bodies were found only in two out of 540 red cells containing such bodies In splenectomized cases *single* HJ bodies thus seem to be spread at random within the population of red cells.

In four of the patients followed during and after splenectomy the first typical HJ bodies were recorded in two cases as early as 6 and 8 hours

*Fig 1* HJ bodies in blood smears. A-G from splenectomized patients. H from a non-splenectomized patient with megaloblastic anemia A The larger body represents the classic type of a HJ body The smaller body in the cell below represents the smallest body to be counted as a HJ body in the present paper B Polycyclic HJ body C "Laced" HJ bodies. D E, F and G represent distinctly extracellular bodies, apparently identical with the "intracellular" HJ bodies which were numerous in these smears (indication for splenectomy in D traumatic spleen rupture in E thrombocytopenia, in F and G hereditary spherocytosis) May-Grunwald-Giemsa original magnification × 1000 approximate magnification on paper in this and following figures see 10 $\mu$ scale


10 µ
A
10 µ
B
10 µ
C
10 µ
D
10 µ
E
10 µ
F
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G
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H

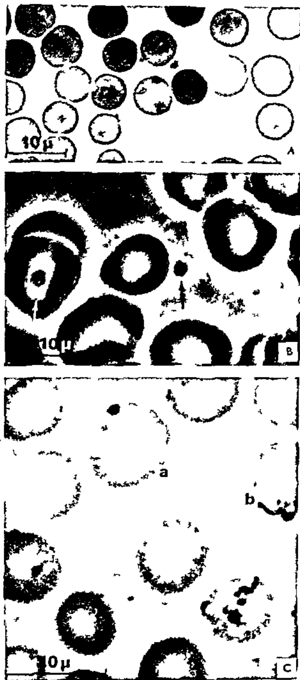



*Fig 2* HJ body like structures. (A) from a splenectomized patient. Wet preparation stained for reticulocytes with brilliant cresyl blue (B) From a splenectomized patient Native blood, unstained, phase contrast. (C) Artificial HJ bodies" (*a b*) observed in a normal blood smear after treatment with DNAse No HJ bodies were present in untreated smears. *May-Grunwald-Giemsa*

after operation in the other cases not until the next day 20–24 hours after operation

It was soon realized that most HJ bodies are typical (Fig 1) only when they are looked for in the classic specimen the air dried blood smear conventionally stained In specimens prepared otherwise this morphologic specificity proved precarious and it was a main problem to establish the identity of bodies visualized by other methods with HJ bodies By experience we learnt however that all HJ bodies are not simply dark circular *dots in red cells many of them could be identified* by more specific structural features A common attribute was a short poorly stained tail sometimes containing a small satellite (Fig 1 C) another helpful marker was a polycyclic contour making the HJ body appear as two bodies fused together (Fig 1 B)

Using these criteria true HJ bodies found after splenectomy proved Feulgen positive and stainable with methyl green In smears fixed with methyl alcohol and exposed for 4 hours to DNAse HJ bodies were absent—*if the smears had been thoroughly washed afterwards* Otherwise DNA fragments from leukocyte nuclei remained in the smear and sometimes closely mimicked HJ bodies (Fig 2 C)

In blood smears from splenectomized patients bodies indistinguishable from HJ bodies were regularly found also *outside* the red cells sometimes sticking to the membrane of red cells sometimes appearing as quite free bodies in the smear (Figs 1 D G) Such bodies should of course *by definition* not be called HJ bodies A definition is however always something of an artefact The HJ body is a small dark dot which attracts attention when seen within the area of a red cell When

*Fig 3* Erythroblast focus in bone marrow smear from a case of hemolytic anemia A nucleophage" phagocyte in the center (*N*) Phagocytic activity still not evident. May Grunwald-Giemsa

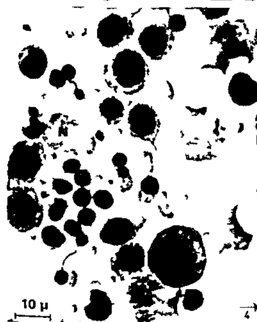
*Fig 4* Erythroblast focus in bone marrow the same case as in Fig. 3 The central nucleophage (*N*) contains 10 still not degraded normoblast nuclei. May-Grunwald Giemsa.

*Fig 5* Nucleophage" in bone marrow smear from a case of idiopathic thrombocytopenia Numerous nuclear remnants in the cytoplasm May-Grunwald Giemsa

*Fig 6* HJ body (*b*) in bone marrow smear from a case of rheumatoid arthritis, without HJ bodies in blood. Note the hiatus" in size between the normoblast nucleus (*a*) and the HJ body (*b*) which in the bone marrow is bridged by nuclear remnants of transitional dimensions in nucleophages" (*N*) May-Grunwald-Giemsa.


N
10 μ
3


N
10 μ
5


N
10 μ
4


a
N
b
10 μ
6

found outside it may easily be discarded as dirt on the glass

If smears containing HJ bodies are rich also in similar extracellular bodies the suspicion must arise that true HJ bodies might also be extracellular bodies localized *on* rather than *within* the red cells For reasons to be accounted for later we found this question of the true position of HJ bodies extremely important but difficult to answer at least in ordinary blood *smears with thin flat* erythrocytes We devoted much attention to this question and found that direct observation gave little evidence for an intracellular position but now and then clear support for the concept of a position outside the cell membrane In thick erythrocytes the focus levels of HJ bodies and cell *margin are regularly slightly different* and *HJ* bodies near the margin of the cell often bulge out a bit from the marginal contour Strong support for this was found in a splenectomized case of familial spherocytosis In the spherocytes of this case suggestive pictures were often seen indicating that the typical position of HJ bodies in this case was *outside* the membrane (Fig 1 G)

In smears stained for proteins (bromphenol blue sin) *intra*cellular HJ bodies might be expected appear as clear spots *in the heavily stained he*oglobin mass Such negative bodies approaching the size of a HJ body were not exceptional in the smears studied by us but they occurred also in the control smears and they never took stain if a nuclear stain was added (neutral red methyl green) Instead we once saw a true HJ body take *stain in a neighbouring red cell where no nega*tive" body had been present while the clear spot under observation remained unstained

Attempts to locate HJ bodies in histologic sections of red cells were disappointing Especially in the EM picture suspected bodies with different internal structure were plentiful both within and outside *the red cells but they were all seen also* in the controls and definite identification of any of these structures with HJ bodies seemed impossible

Not less disappointing were the studies in wet preparations Suspected more or less refringent bodies were often seen within red cells (they were noted already by Howell) *but they were seen also* in the controls and attempts to identify any of them with HJ bodies were unsuccessful The same *can be* said of extracellular bodies of the type pictured in Fig 2 B which were less common however and mainly seen in splenectomized cases. Different methods of *supravital staining in* wet preparations resulted in rich yields of suspected bodies invariably bound to the membrane of red cells (Fig 2 A) but again they were present also in the controls some of them may have been HJ bodies but definite identification proved very difficult

*We conclude from the observations in peripheral* blood that at least after splenectomy DNA bodies indistinguishable from HJ bodies are common also outside the red cells There is some evidence to indicate that in such cases also the true HJ bodies are in reality situated outside the erythrocyte membrane

### *(b) HJ bodies in the bone marrow*

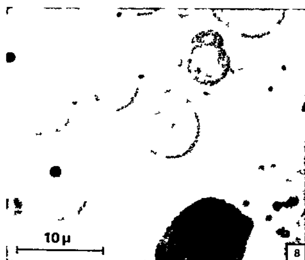
In all the bone marrow smears studied in this series typical HJ bodies were found in various numbers They were especially numerous in conditions of enhanced erythropoiesis and their prevalence seemed to bear no specific relations to the presence or absence of the spleen

It was striking however that even in the bone marrow the hiatus in size between the normoblast nuclei and HJ bodies was not bridged by any nuclear structures of transitional size *within red cells* (Fig 6)

Nuclear fragments similar to HJ bodies were in rare cases seen in *normoblasts* (aberrant chromosomes?) but in most cases (including the splenectomized patients) such fragments in the *normoblasts were virtually absent or at least rather ex*ceptional findings In four specimens the presence of such nuclear fragments in normoblasts was conspicuous (2 cases of megaloblastic anemia 1 case of hemolytic anemia and 1 case with an obscure anemia classified as hypoplastic) Interestingly two of these cases belonged to the group *of four non splenectomized patients in whom HJ* bodies had been found in the peripheral blood A high percentage of the normoblasts in these cases contained *multiple* nuclear fragments (Fig 10)

In the bone marrow true HJ bodies proved often in a conspicuous way to be concentrated to nar*row spots of the smear often together with ap*parently more or less identical bodies lying outside the red cells or just attached to their margins (Figs 7 8) It was often clearly evident that the










*Figs 7 and 8* HJ bodies and similar extracellular bodies in bone marrow smears where they are usually seen close to "nucleophages" (represented by a bare nucleus at *a* and part of the cell body at *b*. Fig. 7 from a case of rheumatoid arthritis. Fig. 8 from a case of idiopathic thrombocytic purpura. May Grunwald Giemsa.

*Fig 9* HJ bodies and similar extracellular bodies in a spleen aspirate with myeloid metaplasia. An endothelial phagocyte at *c*. From a case of myelosclerosis. May Grunwald Giemsa.

*Fig 10* Nuclear fragments in normoblasts. Bone marrow smear from a case of megaloblastic anemia. This probably represents an exceptional formation mechanism for structures appearing as HJ bodies in blood. It is reasonable to assume that HJ bodies in non splenectomized patients, which are often multiple (see Fig. 1 H) belong to this category. May-Grunwald-Giemsa.

groups of HJ bodies and obviously related extra cellular bodies represented nothing else than nuclear debris released from more or less disintegrated endothelial phagocytes which could often be identified in the center of the group

A first thought would be of course to discard such findings as smear artefacts but undoubtedly most of the bodies seen "within" red cells had all the characteristic features of HJ bodies in peripheral blood In addition it is by no means evident that these phagocytes had burst only because of the rough handling in a smear preparation. Most bone marrow cells including *some* phagocytes endure this treatment rather well the burst phagocytes were probably also in vivo brittle cells and it is well known that the fate of "macrophages" is often a rapid disintegration when they have become well filled up with phagocytized material The zone of corpuscular nuclear debris surrounding a burst phagocyte may well represent an in vivo phenomenon.

Numerous unsevered phagocytes containing nuclear debris offered another contribution to the present problem inasmuch as they presented a rich assortment of nuclear bodies intermediate size between normoblast nuclei and HJ bodies these phagocytes, and nowhere else in the bone w the "missing links" bridging this "hiatus" ere present in abundance (Figs. 4 5 6) It should be stressed however that the *individual* phagocyte usually contains nuclear residues of *one* standard size Nuclear residues representing approximately the size of a HJ body proved especially common We got the impression that the degradation process of phagocytized nuclei is for some time at least, stopped up at this stage

We conclude that HJ bodies are normally present in the bone marrow and that only their presence in peripheral blood is related to conditions of asplenia. Observations in the bone marrow suggest that most HJ bodies are nuclear debris resulting from a degradation process in bone marrow phagocytes and only secondarily attached to red cells.

(c) *HJ bodies in spleen aspirates*

HJ bodies were found in two of the spleen aspirates, both representing the cytologic picture of a massive myeloid metaplasia in the remaining eight specimens all *without* a notable admixture of myeloid cells, no HJ bodies were seen In the specimens with myeloid metaplasia they were present in abundance and with a distribution within the smears closely similar to the one described in the bone marrow i.e. concentrated to restricted areas surrounding more or less well preserved phagocytic cells (Fig. 9) Extracellular bodies apparently identical with the HJ bodies were in the spleen still more numerous than in the bone marrow

Considering the high number of HJ bodies in spleen aspirates with myeloid metaplasia it was somewhat surprising to realize that no HJ bodies were found in blood smears from these patients Th spleen may thus be highly efficient in retaining nuclear debris of this size even when they are formed in the spleen itself

## DISCUSSION

HJ bodies are thus normally present in the bone marrow and probably in any tissue where an active erythropoiesis is going on. This observation is by no means surprising it simply supports the assumption generally accepted that the role of the spleen in relation to HJ bodies is simply to remove them from circulating blood.

Our observations in bone marrow do not agree however with the accepted idea that the HJ body is an intracellular remnant of the normoblast nucleus once present in the individual red cell. They strongly suggest instead, that HJ bodies are residues of the digestion of nuclei in bone marrow phagocytes (for obvious reasons in most cases probably extruded normoblast nuclei) The classic HJ body in peripheral blood should thus be an *extra*cellular structure which in asplenia is usually found attached to red cells

Two problems related to HJ bodies may in this way find a satisfactory explanation (i) it is easy to understand that the spleen can rinse peripheral blood from HJ bodies which are in principle extracellular nuclear debris sticking to the red cells if there is no spleen to catch them up (ii) the curious "hiatus" in size between normoblast nuclei and HJ bodies is bridged by nuclear residues of intermediate size found in the phagocytes.

Our conception is thus at variance with the traditional idea that the HJ bodies in blood lodge *within* the red cells It is difficult to define exactly the position of HJ bodies in relation to the erythrocyte membrane but in our material most observa

tions were compatible with the assumption of an extracellular position, whereas convincing evidence for an intracellular position was difficult to obtain

The observations here touch the old problem of how normoblasts lose their nuclei It is nowadays generally accepted that the nuclei are extruded according to the interesting observations of Campbell (2) the denucleation may be an active performance of bone marrow phagocytes which pinch off a part of the cell body containing the pyknotic nucleus from the mature normoblasts This fits well with experience from bone marrow cytology where free nuclei are surprisingly scarce also during enhanced erythropoiesis but phagocytes containing nuclear debris all the more numerous Little is known regarding the order of events in the degradation process of the nuclei evidently a performance which must be accomplished in a short time For our hypothesis to hold good it must be assumed that the nuclear residues may get out of the phagocytes when they have reached the size of a HJ body At present nothing is known regarding the occurrence of such a phenomenon in vivo

An active denucleation of normoblasts is well compatible with the suggestion of Discombe (3) that HJ bodies may represent aberrant chromosomes which as free nuclear fragments may escape the activity of the nucleophage phagocytes In most bone marrow smears in the present series (including all the splenectomized cases) no free nuclear fragments were found in the normoblasts or they were at least too rare to explain the presence of HJ bodies in the red cells It is interesting to note however that among four cases where free nuclear fragments were conspicuous in the normoblasts and often multiple two belonged to the group of four patients in whom HJ bodies were found in blood erythrocytes—again often multiple—in spite of the presence of a presumably functioning spleen It is tempting to assume that *these* HJ bodies were true *intra* cellular nuclear remnants which may have escaped detection by the spleen

The single dark dots in red cells which are traditionally labelled HJ bodies may thus be a bit more heterogenous than they look Observations reported in this paper argue however for the conception that most HJ bodies and especially those seen in asplenia are residues of the degradation of nuclei in bone marrow phagocytes released to the circulation as free bodies with a strong tendency to become attached to the surface of red cells

## ACKNOWLEDGEMENT

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## REFERENCES

1 Bessis, M Cytology of the blood and bloodforming organs. Grune & Stratton New York 1956
2 Campbell, F R. Nuclear elimination from the normoblast of fetal guinea pig liver as studied with electron microscopy and serial sectioning techniques. Anat Rec 160 539 1968
3 Discombe G L origine des corps de Howell Jolly et des anneaux de Cabot. Sang 19 26-, 1948
4 de Gruchy G C. Clinical haematology in medical practice second ed. Blackwell, Oxford 1964
5 Hirschfeld, H. & Dunner L Zur Diagnose und Pathogenese der Milzatrophie. Z. klin. Med. 1 5 536, 1933
6 Howell, W H The life history of the formed elements of the blood, especially of the red blood corpuscles. J Morph. 4 97 1891
7 Jolly J Sur l evolution des globules rouges dans le sang des embryons de mammifères. C R. Soc Biol 58 593 1905
8 — Traite d'hematologie p 76 Maloine & Fils Editeurs, Paris 1923
9 Reimann, F Uber das Auftreten basophiler auf den Erythrocyten aufgelagerter Korpuskel im Blute nach Vornahme einer Splenektomie Acta med. scand. Suppl 445 358 1967
10 Schur H. Uber eigenartige basophile Einschlusse in den roten Blutkorperchen. Wien med Wschr 58 441 1908

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# METABOLISM AND DISTRIBUTION OF IgG IN PATIENTS CONFINED TO PROLONGED AND STRICT BED REST

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*Abstract* The metabolism of IgG has been studied in nine patients, who were on long term treatment in bed for cerebral damage and who were practically unable to move The amount of circulating IgG was slightly increased, but IgG catabolism calculated by three methods—the U/P method, whole-body counting, and Matthews method—was significantly increased

The extravascular distribution of IgG was increased and the extravascular/intravascular quotient was 2.0 and differed highly significantly from the quotient of 0 9 in healthy controls.

The cause of the increased metabolism of IgG and the altered distribution are discussed with special reference to the influence of posture on the results.

An earlier study (1) has been made of the IgG metabolism in a group of healthy control subjects carefully examined clinically Different methods were used for determining γ-globulin concentration in serum and for calculating the metabolism The extravascular distribution of IgG was also calculated on the basis of whole body data and the values were compared with those obtained by the conventional retained dose method This study (1) as well as data published by earlier authors (3 4 5) showed that the extravascular and intravascular compartments are equal in size

In most studies of the protein metabolism in pathological conditions the analyses were made in patients treated by bed rest for varying length of time The question whether the bed rest might have played a part in causing the recorded changes is not discussed in any study To investigate this problem we have chosen a group of long term bed patients with cerebral vascular disease With one exception all of them were virtually unable to move The object of this choice of patients was to obtain clear cut data that would throw light on the question whether bed rest alone can cause disturbances of protein metabolism Obviously it is difficult to refine the experimental conditions and the primary disease in these cases cerebral insult, may of course influence the results It is however a great problem to obtain a representative series of healthy controls confined to strict bed rest during the long period of time required to carry out the study The results achieved show not only some metabolic disturbances but also a distinctly changed IgG distribution with a marked increase of the extravascular pool The clinical significance of these findings will be discussed here

## MATERIAL

Nine patients with cerebral vascular disease (cerebral haemorrhage or thrombosis) were studied Their ages ranged from 67 to 88 years. None had anaemia The haematocrit values varied between 35 % and 47 All had normal creatinine values and only slight proteinuria now and then As a catheter à demeure had to be used, urinary tract infection caused by E coli was present in all cases. The ESR was slightly to moderately elevated (13–65 mm) in most Eight of the patients were wholly unable to move in bed and had to be turned over regularly Patient no 9 was occasionally able to sit in a chair for a while

## METHODS

Isolation and labelling of IgG quantitative determination of proteins, and the isotope studies were performed according to methods described earlier (1)

Table I *Composition and age of the material*

MS = mitral stenosis ASD = atrial septal defect ASHD = arteriosclerotic heart disease

| | No of pats | Age (y) | |
|---|---|---|---|
| | | Mean | Range |
| VOC | | | |
| MS | 3 | 49 | 43–56 |
| Combined valvular lesions | 6 | 48 | 34–61 |
| ASD | 8 | 40 | 23–62 |
| ASHD | 7 | 65 | 53–78 |
| Varia | 5 | 42 | 19–65 |

Table II *Long term results in the entire study*

Abbreviations as in Table I Numbers within brackets = patients at risk

| | Total no of pats | No of pats still in sinus rhythm after | | | | |
|---|---|---|---|---|---|---|
| | | 1 week | 3 mo | 6 mo | 12 mo | 24 mo |
| VOC | 17 | 16 | 12 | 12 | 10 (16) | 6 (14) |
| ASHD | 7 | 6 | 5 | 5 | 5 (7) | 3 (5) |
| Varia | 5 | 3 | 3 | 2 | 2 (5) | 1 (4) |
| Total | 29 | 25 | 20 | 19 | 17 | 10 |

visible despite the small groups namely that e sinus rhythm is maintained longer in patients with short duration of flutter prior to the conversion The recurrence frequency does not appear to be dependent on diagnosis (Table II) age (Table III) or heart size (Table IV) which, on the whole is in agreement with the corresponding conditions for atrial fibrillation (3 5)

*Complications*

One case of asystole occurred but after immediate heart massage and adequate ventilation pronounced sinus bradycardia first ensued later the rhythm was normalized without further measures This patient was 78 years old and the oldest in the series Digitalis was withdrawn in the usual way 48 hours before the conversion Six patients had nodal rhythm which changed to sinus rhythm in three cases without any additional measures In one case pulmonary edema developed in connection with the nodal rhythm This was repeated at a later regularization effort One patient who had nodal rhythm after regularization developed about 3 hours later a definite myocardial infarction complicated by further arrhythmias Finally in one case with a plausible familial cardiomyopathy nodal rhythm developed in connection with a pronounced fall in blood pressure which was complicated by anuria after 48 hours Shortly after the atrial flutter of the patient recurred with an obvious improvement of the general condition and normal urine production as a consequence. In four of the six cases with nodal rhythm the patient was treated with digitalis and/or quinidine at the moment of conversion One patient devel

Table III *Long term results in relation to age of the patient*

Abbreviations as in Table I

| Age (y) | Total no of pats | No of pats still in sinus rhythm after | | |
|---|---|---|---|---|
| | | 3 mo | 12 mo | 24 mo |
| 15–35 | 7 | 3 | 3 | 2 |
| 36–55 | 10 | 9 | 7 | 4 |
| 56– | 12 | 8 | 7 | 4 |
| Total | 29 | 20 | 17 | 10 |

Table IV *Long term results in relation to heart volume*

Abbreviations as in Table I

| Heart volume ml/m² BSA | Total no of pats | No of pats still in sinus rhythm after | | |
|---|---|---|---|---|
| | | 3 mo | 12 mo | 24 mo |
| < 500 | 8 | 6 | 4 | 2 |
| 500–650 | 12 | 7 | 7 | 5 |
| > 650 | 8 | 7 | 6 | 3 |
| Total | 8 | 0 | 17 | 10 |

ᵃ One patient s heart volume not known

Table V *Long term results in relation to duration of atrial flutter*

Abbreviations as in Table I

| Duration | Total no of pats | No of pats still in sinus rhythm after | | |
|---|---|---|---|---|
| | | 3 mo | 12 mo | 24 mo |
| < 3 weeks | 10 | 6 | 6 | 3 |
| 3 weeks–6 mo | 11 | 10 | 8 | 5 |
| > 6 mo | 8 | 4 | 3 | 2 |
| Total | 29 | 20 | 17 | 10 |

oped a supraventricular tachycardia after the regularization with a frequency of 150 lasting for seven days whereupon the flutter recurred

## DISCUSSION

Flutter is a rather uncommon heart disease compared to atrial fibrillation Thus Katz and Pick (10) found 270 cases with atrial flutter and 5859 cases with atrial fibrillation in a series of 50 000 patients

Atrial flutter is usually combined with organic heart disease In a series including 104 patients all except one had a chronic organic cardiopathy (6) However occasionally more acute conditions may be present such as rheumatic fever diphtheria hyperthyroidism and coronary artery occlusion (4) In this connection it should be mentioned that already in 1950 i e before the establishment of intensive coronary care for infarctions Hejtmancik et al (7) observed seven cases of atrial flutter among 82 with acute myocardial infarction Reasonably this figure ought to increase in connection with modern coronary care

Previously it has always been difficult to treat flutter whereas regularization from the hemodynamic point of view has been desirable especially in cases with low or varying blocks Almost exclusively digitalization, occasionally in combination with quinidine was the former practise As a rule considerably larger doses of digitalis than otherwise were then required with increased risks of complications The titration of this dose and sometimes the institution of quinidine thus required a considerable time In a number of early but rather large series the reversal frequency has varied between 45–60 (8 14) The tendency to recurrence was large Ever since the introduction of DC regularization a simple rapid and effective method of treatment has been offered

Against the background of the earlier therapeutical difficulties we considered it to be of interest to study not only the conversion frequency but also the long term results and the complications Patient series with merely flutter have very seldom been studied from these points of view As a rule the investigations have been limited to putting the flutter patients into the same group as the fibrillation patients This mode of action is questionable as is demonstrated in Table II where the long term result diverges from that in a fibrillation material (3) Thus no less than 63 % of the patients retain their sinus rhythm after one year corresponding to 25 % in the fibrillation group Consequently the immediate conversion frequency as well as the long term results are more favorable than those in the fibrillation material As regards the effects of heart volume and flutter duration on the long term results these agree with those current in cases of fibrillation (3 5) Against these favorable results must be placed the remarkably high frequency of complications which supports the clinical observation that chronic atrial flutter is mostly an expression of a more pronounced cardiopathy with a more defective sinus node Our definite impression is that the P–Q time has been longer after regularization in this material compared to earlier fibrillation materials This should still further confirm the statement of more pronounced heart disease in cases of atrial flutter It is of interest that there was one case of asystole in this small material which has previously been reported in connection with conversion of atrial flutter but is extremely uncommon in regularization of atrial fibrillation Also in Bjerkelund and Orning s series of DC shock treated atrial arrhythmias (5) one of the most dramatic episodes of complicating arrhythmias after shock treatment occurred in a patient with atrial flutter

The predominant complication appears to be nodal rhythm It is striking that the nodal rhythm has been badly tolerated by the patients and moreover has caused additional severe complications The possibility that digitalis as well as quinidine have increased the risk of nodal rhythm is obvious In our opinion these drugs ought to be withdrawn in adequate time prior to DC shock (2 12)

## CONCLUSION

Regularization of atrial flutter has previously often been a difficult and time-consuming clinical problem with only moderately successful treatment results DC conversion has considerably altered this picture partly because of the very high conversion frequency and partly with respect to a reasonable long term result We emphasize the relatively high frequency of complications which however must be viewed in the light of the fact that the patients usually have severe heart disease

## REFERENCES

1 Aberg, H. & Cullhed, I Direct current countershock complications Acta med scand. 183 415 1968
2 Aberg, H Atrial fibrillation. A review of 463 cases from Philadelphia General Hospital from 1955 to 1965 Acta med. scand 184 425 1968
3 Aberg, H & Cullhed I Direct current conversion of atrial fibrillation—long term results. Acta med. scand 184 433 1968
4 Bellet, S Clinical disorders of the heart beat 2nd ed., pp 144–176 Lea & Febiger Philadelphia 1963
5 Bjerkelund C & Orning, O M An evaluation of DC shock treatment of atrial arrhythmias Acta med scand 184 481 1968
6 Gillman, H. & Preiss H. Vergleichsuntersuchungen an 104 Patienten mit Vorhofflattern. Z. Kreisl Forsch 52 139 1963
7 Hejtmancik, M R., Herrmann G R. & Bradfield J Y Atrial flutter Amer Heart J 40 884 1950
8 Herrmann, G R. & Hejtmancik, M R. Treatment of atrial flutter Amer Heart J 41 182, 1951
9 Jonsell S Method for the determination of the heart size by teleroentgenography (A heart volume index.) Acta radiol (Stockh) 20 325 1939
10 Katz, L. N & Pick, A. Clinical electrocardiography I The arrhythmias. Lea & Febiger Philadelphia 1956
11 Lown, B Amarasingham, R. & Neuman J New method for terminating cardiac arrhythmias J Amer med. Ass 182 548 1962
12 Lown B Kleiger R. & Williams J Cardioversion and digitalis drugs changed threshold to electric shock in digitalized animals Circulat. Res 17 519 1965
13 Lown, B Electrical reversion of cardiac arrhythmias Brit. Heart J 29 469 1967
14 Parkinson, J & Bedford D E. Course and treatment of auricular flutter Quart. J Med 21 21 1927

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# ONE YEAR S EXPERIENCE OF MEDICAL INTENSIVE CARE UNITS

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*Abstract* At Ullevaal Hospital the three medical departments have separate intensive care units which have been similarly designed and are similarly run. The units include 30–36 beds serving a total of about 300 medical beds Equipment is available for simultaneous monitoring of 18 patients by a combination of bedside oscilloscopes and alarm systems and a central observation station

In the first year a total of 1801 patients were treated in the units 613 patients had acute myocardial infarction 589 other heart diseases, 178 intoxication 109 gastrointestinal hemorrhage 114 acute or chronic respiratory failure and the rest were observed for different reasons

Of 613 patients with acute myocardial infarction 150 died (24 ) Circulatory arrest occurred in 118 patients (21 survived) cardiogenic shock in 25 (6 survived) and pulmonary edema without cardiogenic shock in 42 (27 survived) Arrhythmias of clinical importance were recorded in 339 (55 ) of the patients with acute myocardial infarction A–V block of second and third degree occurred in 60 patients (10 ) 45 of whom were treated with pacemaker

The first years experience has proved the usefulness and value of these combined medical intensive care units The most important advantages have been 1) an effective and continuous round-the-clock observation 2) a medical staff available at any time and well trained in the use of the specialized equipment needed for immediate treatment and 3) a considerably improved opportunity to gain experience in the treatment of acutely ill medical patients.

There has been a growing interest in intensive care units during the last years The three medical departments at Ullevaal Hospital receive about 5500 acutely ill patients every year The problems of management of these patients have made it ever more necessary to have special units for intensive care In each of the departments therefore a modern intensive care unit has been established There has been close cooperation between the three units and the therapeutic procedures staffing admission and discharge policy have been similar in the three wards. The units were planned as medical intensive care units not only as coronary care units

This report presents a description of the units and the experience gained after one year

Fig. 1 shows the design of one of the units. Each unit has twelve beds, of which usually not more than ten have been occupied at any time From a central station all except two beds can be directly observed Special equipment includes six mobile cardioscopes with heart rate meter and rate activated alarm in each unit Monitoring of both ECG and pulse is possible but usually only the ECG monitoring is used The monitor produces a visual alarm in the patients room and both visual and auditory alarm at the central station Two 3-channel oscilloscopes for continuous ECG monitoring of six patients simultaneously are placed in the central station, which also has a 2-channel electrocardiograph which automatically records the ECG when the alarm is started A memory loop is available for one of the channels

Oxygen and suction points are mounted on the wall by the patients bed Further equipment includes DC defibrillators intermittent positive pressure respirators equipment for endotracheal intubation and for insertion of pacemaker electrodes

### *Admission and duration of observation in the units*

In the period from October 1967 to September 1968 a total of 1801 patients were treated in the medical intensive care units, i e 30 % of the total number of patients admitted to the medical departments in the same period The reasons for admission are seen in Table I and the ultimate diagnoses in Table II

In 600 (61 %) of the patients who were admitted for acute myocardial infarction the di

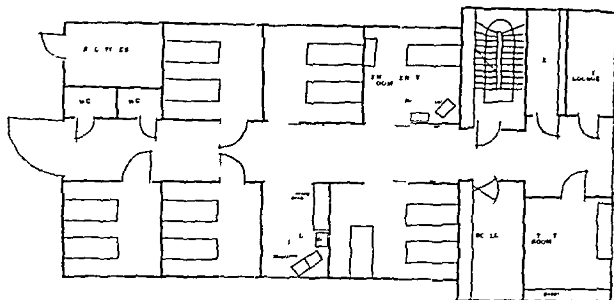


*Fig 1* Floor plan of one of the medical intensive care units. All patients except two may be directly viewed from the central nurse's station through windows and glass doors (stippled in the diagram) In this unit a special emergency room is used for defibrillation, in sertion of pacemaker electrodes and other special procedures. The stairs connect the three units, which are placed in the same part of the building but on different floors.

agnosis were verified, in 267 (27%) a diagnosis of coronary heart disease without acute myocar ial infarction was made and in 115 (12%) no oronary h art disease was found Sixty patients in th last group had oth r cardiac or pulmonary diseas s while 55 pati nts had no cardiopulmonary dis as A diagnosis of acute myocardial in farction was made in an additional 13 patients who were admitted to the units under oth r di agnoses Pati nts with acute myocardial infarction were usually observed in the units for 4–5 days, and 60% were transf rred to the g n ral wards within 10 days

Table I *Reasons for admission*

| | No of pats |
|---|---|
| Suspected myocardial infarction | 982 |
| Chronic heart disease with different compl cations | 313 |
| Unconsciousness | 206 |
| Intoxication | 51 |
| Gastrointestinal hemorrhage | 114 |
| Respiratory failure in pulmonary disease | 69 |
| Other reasons | 66 |

Table II *Ultimate diagnoses*

| | No of pats |
|---|---|
| Acute myocardial infarction | 613 |
| Other heart diseases | 389 |
| Intoxication | 1 8 |
| Gastrointestinal hemorrhage | 109 |
| Respiratory failure in pulmonary diseases | 114 |
| Neurological diseases | 56 |
| Diabetes | 20 |
| Renal diseases | 8 |
| No organic disease found | 114 |

In 271 (86%) of the patients who were ad mitted for chronic heart dis ase th diagnoses was verifi d Most of the r maining 42 pati nts had pulmonary diseases

Of 206 patients admitted for unconsciousn ss, 127 were intoxicated In addition 51 pati nts were treat d in th units for various intoxications without unconsciousness. Cerebral stroke was the cause of unconsciousness in 44 patients coma diabeticum in 11 and heart or lung diseas s in 24 patients

In 109 (96%) of the patients who w re ad mitted for gastrointestinal hemorrhage the diagnosis was verifi d.

Respiratory insufficiency in pulmonary dis ase was the caus of admission in 69 patients but was present in an additional 45 who were admitted under other diagnoses.

The majority (76%) of the patients were transferred to general medical wards 4% of the transferred patients were later readmitted to the intensive care units. Of the intensive care patients 10% died in the units 6% were moved to other specialized wards (surgical, psychiatric) and 8% were discharged directly from the units Fifty per cent of the patients were transferred from the units to other wards or discharged within 3 days and 75% within 6 days of admission A few patients were treated for longer periods (maximum 36 days)

## ACUTE MYOCARDIAL INFARCTION

### *Frequency and Mortality*

The diagnosis of acute myocardial infarction was based upon 1) typical ECG changes 2) suggestive ECG changes accompanied by transient rise in the SGOT or 3) autopsy findings. A total of 801 patients with myocardial infarction were admitted to the medical departments during the one year period 287 (36%) of whom died. Ninety two patients (11%) were however dead on arrival or died shortly after hospitalisation never reaching the units. Of the remaining patients 613 were observed in the units and 96 in the general wards The latter group included 45 patients who were treated for other diseases myocardial infarction being diagnosed at autopsy and 51 patients without serious complications who arrived at the hospital more than 4–5 days after the attack

Of 613 patients with myocardial infarction treated in the units 51 died during the first day and 33 during the next 4 days Sixty six patients died later than the 5th day after admission 28 while they still were in the units, and 38 after they had been transferred to the general wards.

The myocardial infarction mortality rate was 18% in patients treated in the intensive care units, and 24% when deaths in the general wards are included The mortality was 21% in patients who arrived within 6 hours after the coronary attack, and 10% in patients who arrived later

### *Time of Arrival in Hospital Delaying Factors*

318 patients were asked to estimate the time interval between the onset of symptoms and their arrival in hospital The results are seen in Fig. 2.




*Fig 2* The number of patients with acute myocardial infarction in per cent at different times from onset of symptoms to the call for help (x——x) and to arrival in hospital (x-- x)

36% of the patients arrived within 3 hours, 65% within 6 hours while 9% were delayed more than 48 hours. 92 of 318 patients were asked about the mode of admission 78% had called their own doctor or Oslo Medical Emergency Centre ("Legevakten") 15% of the patients came directly to "Legevakten" from where they were referred to the hospital and 7% came directly to the hospital

Most of the delay before hospitalization was caused by the patient waiting to call for medical assistance after the symptoms had started (Fig 2) Only 50% of the patients had called for a doctor within 110 min 80% within 6 hours The doctor called up the ambulance within 45 min after he got the message to see the patient in 50% of cases, and within 80 min in 80% of cases. 50% of the patients came to hospital within 30 min after the ambulance was called for 80% within 45 min

### *Complications*

Circulatory arrest, cardiogenic shock, or pulmonary edema were seen in 185 patients (Table III) In 84 patients only one of these complications was seen. In 101 patients more than one complication was seen at the same time. In these cases we often found it difficult to tell which was the primary and which was the secondary complication. Some cases labelled cardiac collapse might equally well have been called cardiogenic shock. In Table III the diagnoses cardiac shock and pulmonary edema refer only to cases in

Table III *Severe complications in 613 patients with acute myocardial infarction*

| | No of cases | Short time survival | Long time survival (left hospital alive) |
|---|---|---|---|
| *Circulatory arrest* (118 patients) | | | |
| Ventricular fibrillation "primary" | 29 | 21 | 16 (55 ) |
| Ventricular fibrillation secondary to shock or pulmonary edema | 32 | 4 | 1 (3 ) |
| Asystole cardial collapse | 53 | 4 | 4 (7 ) |
| Cardiac rupture | 4 | | |
| *Cardiogenic shock* (25 patients) | | | |
| As the only complication | 13 | | 5 (38 ) |
| Associated with pulmonary edema | 12 | | 1 (8 ) |
| *Pulmonary edema* (54 patients) | | | |
| As the only complication | 42 | | 27 (64 ) |

which these complications were not secondary to circulatory arrest or severe arrhythmias

*Circulatory arrest* was seen in 118 patients (19%) In 29 cases this was due to primary ventricular and in 21 defibrillation was success- five however died later in the hospital In patients ventricular fibrillation was seen secondary to other serious complications such as pulmonary edema and cardiogenic shock. Only one of these patients left the hospital alive In 53 patients the cause of circulatory arrest was cardiac asystole (2 survived) or cardiac collapse (2 survived) Four patients had cardiac rupture

Of the patients with circulatory arrest 21 left the hospital alive On of them died 5 months later the others are still alive but two have signs of severe cerebral damag

*Cardiogenic shock* i.e systolic arterial pressure less than 80 mm Hg together with characteristic clinical signs of shock, was seen in 25 patients (4%) In 13 patients it was seen as the only serious complication (5 survived) in 12 patients it was combined with pulmonary edema (1 survived) Cardiogenic shock secondary to circulatory arrest was seen in several patients, as has been mentioned above

*Pulmonary edema* was seen in 54 patients (9%) as the only complication in 42 (27 survived)

The simultaneous occurrence of two or more of the above mentioned complications was associated with a very bad prognosis. Of 84 patients with a single complication 48 survived while only six survived of 101 patients with more than on complication

*Arrhythmias* All arrhythmias of clinical importance were recorded. Less severe and short lasting arrhythmias may however have been overlooked 45% of the patients had no arrhythmias according to the criteria used here (Table IV) Of patients admitted to the hospital within 6 hours after the onset of symptoms arrhythmias were found in 68%

The high mortality in the patients with sinus tachycardia is due to a high percentage of cardiogenic shock *in this group Ventricular fibrillation* and A–V block were also associated with a high mortality The other arrhythmias were not associated with increased mortality when they were seen alone Irrespective of the type of arrhythmia the mortality was 21% when a single type was

Table IV *Cardiac arrhythmias in 613 patients with acute myocardial infarction*

| Type of arrhythmias | No | As the only or most serious arrhythmia* |
|---|---|---|
| Sinus tachycardia (> 120 min) | 37 | 23 (11) |
| Sinus bradycardia (needed treatment) | 26 | 15 (1) |
| Nodal rhythm | 38 | 4 (0) |
| Multiple ventricular ectopics (1 in 10) | 151 | 68 (4) |
| Atrial fibrillation or flutter | 74 | 50 (1) |
| Supraventricular tachycardia | 27 | 1 (0) |
| Ventricular tachycardia, short ( 30 sec) | 44 | ? (0) |
| Ventricular tachycardia (> 30 sec) | 13 | 4 (1) |
| Ventricular fibrillation short (spontaneous conversion) | 5 | (1) |
| Ventricular fibrillation | 61 | 61 (44) |
| Sino-auricular block | 15 | 10 (0) |
| Atrio-ventricular block 1 | 12 | 3 (0) |
| Atrio-ventricular block 2 –3 | 60 | 60 (23) |
| No arrhythmias | 274 (30) | |
| A single type of arrhythmia | 186 (39) | |
| Two types of arrhythmias | 86 ( 1) | |
| Three or more types of arrhythmias | 67 (22) | |

* Within brackets number of patients who died

present 24% when two types were present and 33% when three or more types were present

*A–V block* Sixty patients had A–V block of second and third degree in 46 of whom the block was complete One of the patients had block prior to the myocardial infarction in the others the block was probably directly related to th infarction Transvenous insertion of pacemaker electrode was performed as described in a previous paper (2) Forty five patients were treated with pacemaker in 14 cases because of Adams Stokes attacks (7 survived) and in 12 cases because of bradycardia (5 survived) In 19 cases the pacemaker electrodes were inserted prophylactically three of these patients later died from other complications In six patients the pacemaker probably caused ventricular fibrillation An immediately successful defibrillating shock was given to all of them and further complications were not seen

In 15 patients with A–V block pacemaker was not used In five patients the condition was terminal and considered hopeless they all died One patient refused pacemaker In nine patients with partial A–V block most of them with Wenckebach periods and with a satisfactory heart rate prophylactic insertion of pacemaker was found unnecessary They were all however carefully monitored and had an intravenous catheter inserted All th se patients survived

## OTHER HEART DISEASES

589 patients with chronic coronary or other heart disease were observed in the intensive care units 327 because of chest pains Twelve patients had ventricular fibrillation two were successfully defibrillated but only one was discharged alive Thirty six patients were admitted b cause of Adams Stokes attacks due to chronic A–V block 111 patients had other arrhythmias and some of them were treated with electroshock because of ventricular tachycardia or atrial fibrillation or flutter Eighty two patients suffered from pulmonary edema without acute myocardial infarction Five of these patients died The remaining 21 patients were treated in the units for different cardiac reasons

## INTOXICATIONS

178 patients of whom 127 were unconscious on admission were treated in the units Two patients died Twenty three patients were treated with endotracheal intubation 11 suffered acute respiratory arrest all survived Seven patients were treated with osmotic diuresis 63 % of the patients were transferred from the unit on the day after admission and only four stayed more than 6 days One third were discharged directly from the units The others were transferred to the psychiatric department or to general wards

## GASTROINTESTINAL HEMORRHAGE

109 patients suffered from gastrointestinal hemorrhage Twenty five patients were shocked 70 had blood transfusion and 33 were transferred to surgical departments

## RESPIRATORY FAILURE

114 patients were treated because of acute or chronic respiratory failure The most common causes were chronic bronchitis emphysema and bronchial asthma Twenty four patients were treated by intermittent positive pressure breathing In 12 of the patients the respiratory failure was acute and immediate intubation and ventilation were necessary Eleven of the 24 patients were treated only for a few days through an endotracheal tube and could leave the hospital days or weeks later The other 13 patients needed a tracheostomy and were ventilated for a longer period only five survived Five patients were in such a bad condition that respiratory treatment was found hopeless they all died in the hospital In the remaining 85 patients artificial ventilation was not necessary and they all survived The total mortality in cases of respiratory failure was 11

Forty four patients with acute respiratory distress secondary to other diseases were also treated in a respirator 12 with subarachnoid or cerebral hemorrhage all of whom died 11 with intoxication all survived 21 with respiratory arrest after cardiac arrest all died

## DISCUSSION

The introduction of the intensive care unit represents a great advance in the treatment of acutely ill patients. The most common type of medical intensive care units has been the coronary unit, and the experiences from several of these have been published (1, 3, 4, 5). Intensive care is not only a question of complex electronic and mechanical equipment, but first and foremost there is a need for a well trained and experienced staff on a round-the-clock service. Patients other than those with acute myocardial infarction may need this service. The units in our hospital were therefore designed for general medical intensive care. The first year's experiences have confirmed the usefulness and value of this type of unit. Although cardiac patients have been the most dominant group, many other groups of patients have been treated in the units. This has probably been of benefit to the patients, just as it has proved valuable to the staff in making the units more flexible and the work and problems of the units more varied than in pure coronary care units. No serious drawbacks have been noted.

We have treated the patients in two-bed rooms; respirator patients and other severely ill pa... have been treated in separate rooms. Care ... been taken not to place patients with acute myocardial infarction together with other severely ill patients, and to avoid as far as possible all excitement and distress. A few patients have been affected by the monitoring equipment, but usually patients as well as relatives have had a very positive attitude to the intensive care units.

Of the methods available for continuous monitoring we have found that ECG with a combination of bedside and central oscilloscopes is the most valuable. A well trained observer does not have to watch the oscilloscope continuously in order to get an impression of the stability of the heart rhythm in the different patients. The "memory loop" has been of some use when the observer has wanted to record intermittent rhythm disturbances of short duration. Pulse monitoring is desirable for patients on pacemaker treatment because the ECG monitor may be triggered by the pacemaker artefact and not give an alarm in cardiac standstill due to pacing failure.

The ideal proportion of beds for intensive medical care related to the total number of medical beds is difficult to assign. In our medical departments in which nearly all patients are admitted as acutely ill patients, one intensive care bed to every ten beds seems to be a reasonable proportion. We have, however, felt the need for an intermediate "secondary intensive care unit" in connection with the units.

The medical staff working in the units must be well trained. After an initial training course continual education of nurses and auxiliary medical staff is imperative. The intensive care units have not resulted in any reduction of the total nursing staff in the medical wards, but the load of work in the general wards has been reduced, especially during the night. The general wards have been relieved of the severe—often impossible—burden of treating a severely ill patient in addition to the ordinary patients.

The value of such units is difficult to prove statistically in terms of reduction of mortality or number of saved lives.

The hospital mortality in acute myocardial infarction depends on how quickly the patients are brought to hospital as well as the criteria used to diagnose myocardial necrosis. The number of lives saved is a credit not only to the intensive care units per se, but also to new equipment and therapeutic methods. In the intensive care units described in this paper 21 patients with acute myocardial infarction have been successfully treated for episodes with circulatory arrest and have left hospital alive. A further number of patients have probably been saved by other therapeutic and prophylactic procedures. In many patients acute respiratory arrest has been successfully treated by intubation and positive pressure ventilation. The prevention of serious arrhythmias by selective prophylaxis in patients with unstable heart rhythm and careful observation and care of patients with severe gastrointestinal hemorrhage, intoxication or lung failure, severe bronchial asthma included, have probably improved the outlook for these patients.

The most important advantages of the medical intensive care units have, in our opinion, been:

1) An effective continuous round-the-clock observation, 2) a medical staff available at any time which is well trained in the use of the specialized equipment needed for immediate treatment, 3) a considerably improved opportunity to gain experience in the treatment of acutely ill medical patients.

## REFERENCES

1 Day H W Acute coronary care—a five year report. Amer J Cardiol. 21 252, 1968
2. Grendahl H & Sivertssen, E. Endocardial pacing in acute myocardial infarction. Acta med. scand. 186 21 1969
3 Lawrie D M., Greenwood T W., Goddard, M Harvey A. C., Donald, K. W., Julian, D G & Oliver M. F A coronary care unit in the routine management of acute myocardial infarction. Lancet 2 109 1967
4 Sandoe E. & Hansen, P F Intens overvågning og behandling af pasienter med akut myocardieinfarct. Nord. Med. 76 1128 1966.
5 Thomas, M., Jewitt, D E. & Schillingford, J P Analysis of 150 patients with acute myocardial infarction admitted to an intensive care and study unit. Brit. Med J 1 787 1968

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# THE EFFECT OF CALCITONIN INJECTED INTO HYPERCALCAEMIC AND NORMOCALCAEMIC PATIENTS

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*Abstract* Porcine calcitonin has been purified by the method of Tenenhouse et al. followed by gel filtration. Forty five MRC units were injected i.m. or i.v. into seven hypercalcaemic and five normocalcaemic patients. Four control subjects received the vehicle for calcitonin

The serum concentrations and the urinary excretion rates of calcium, magnesium, phosphate sodium, potassium, and creatinine were measured at short intervals for three days. The hormone was injected on the second day Complexed and ionized calcium, alkaline phosphatase and protein in serum were determined. The calcium turnover and bone formation rate were determined by the technique of Heaney and Whedon using Ca.

There was a significant hypocalcaemic effect in all patients, most pronounced in the hypercalcaemic group However the effect seemed to be better correlated to the bone formation rate than to the degree of hypercalcaemia.

A significant decrease in serum magnesium concentration was observed in the hypercalcaemic group It was difficult to evaluate the effect on serum phosphate because of large diurnal fluctuations. An increased urinary sodium excretion rate after calcitonin administration was registered.

Porcine calcitonin was first given to human subjects by Milhaud et al (15) Moderate hypocalcaemic responses were registered in the four cases studied A more pronounced effect was reported by Foster et al (7) Calcitonin in doses of 1–22 MRC units was found to lower the serum calcium concentration by 0 6–0 8 mEq/l for periods from 6–18 hours in three patients with hypercalcaemia complicating disseminated malignant disease Bell et al (2) found similar results in three normal human subjects Haas and Dambacher (10) infused 1–10 MRC units into thirteen patients and found the most striking effect in patients with hypercalcaemia Bijvoet et al (4) studied the effect of intravenous injection of calcitonin into three hypercalcaemic and nine normocalcaemic patients These authors suggested that the degree to which calcitonin may lower the serum calcium concentration depends on the rate of bone turnover rather than on the serum calcium level because the most impressive hypocalcaemic response was seen in patients with generalized Paget s disease and in patients with hyperthyroidism, conditions which are usually accompanied by an elevated bone turnover rate However no determinations of bone metabolism were made In the present work we have tried to correlate the hypocalcaemic effect with bone turnover as estimated by [4]Ca studies and by alkaline phosphatase level

In some studies on the effect of calcitonin in man no hypomagnesiaemic effect was demonstrable (2 4 7) Singer et al (18) and Haas and Dambacher (9) however observed a hypomagnesiaemic effect of calcitonin in some patients but no exact data were given In the present investigation a detailed study of the effect of calcitonin on serum magnesium was made

The calcium fractions in serum consisting of ionized complexed and protein bound calcium were determined before and after calcitonin injection in most of the patients in order to evaluate the calcitonin effect on the various fractions

As the reports concerning the net effect of calcitonin in man on the urinary excretion of calcium magnesium sodium and phosphate are controversial (1 4 9 18) these parameters were studied as well

## MATERIAL

The first two patients given calcitonin were suffering from multiple myeloma. Because of pronounced diurnal fluctuations in serum calcium concentration (Fig. 1) they were excluded from the material.

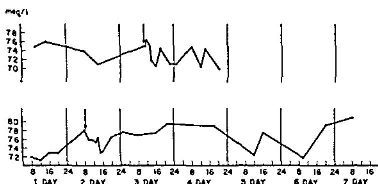



*Fig 1* The effect of calcitonin injection on serum calcium levels in two cases of multiple myeloma. Arrows indicate time of injection

Details of the other patients are given in Tables I and II One of th hypercalcaemic patients (case 1) received an intramuscular and an intravenous calcitonin injection at an interval of six weeks Another patient from the hypercalcaemic group (case 3) had a control injection (the calcitonin vehicle) four weeks after the calcitonin injection.

None of the patients suffered from liver disease

## METHODS

*Calcitonin preparation*

Calcitonin was prepared from pig thyroid glands by the method of Tenenhouse et al (20) including the ion exchange of the trichloroacetic acid precipitate followed by sation The resulting powder was dissolved in M formic acid and subjected to filtration on a × 125 cm column G 75 sephadex fine (Pharma Twenty minute fractions were collected at a flow rate of 25 ml/h The active fractions were pooled and lyophilised This material was dissolved in acid 0 9% sodium chloride (pH 2 0) containing 0 1% human albumin The solution was sterilized by filtration and kept frozen in ampoules at −20 C until use It was tested for bacterial contamination and the pH was adjusted to 6 9 by addition of 1 *N* sodium hydroxide immediately before the injection. The material had a potency of 1 6 MRC (Medical Research Council) units per mg protein, measured as tyrosine (14) The activity was determined by the bioassay procedure of Kumar et al (12) in a four point assay each point representing the mean decrease in serum calcium concentration of six 150 g male Wistar rats fasted overnight The index of precision was 0 15 MRC Research Standard B (lot. no 67/178) supplied by Dr J A Parsons, Division of Biological Standards, National Institute for Medical Research London was used as a standard

A quantity of 0 7 MRC unit was given intracutaneously

Table I *Clinical data*

| | Diagnosis | Weight (kg) | Sex | Age (y) | Creatinine clearance (ml/min) |
|---|---|---|---|---|---|
| *Hypercalcaemic group* | | | | | |
| Case 1 | Parathyroid adenoma (verified by operation) | 35 | ♀ | 57 | 22 |
| Case 2 | Hyperthyroidism | 44 | ♀ | 49 | 63 |
| Case 3 | Parathyroid adenoma | 62 | ♂ | 68 | 70 |
| Case 4 | Lymphatic leukaemia | 58 | ♀ | 68 | 59 |
| Case 5 | Idiopatic hypercalcaemia | 60 | ♀ | 57 | 93 |
| *Normocalcaemic group* | | | | | |
| Case 6 | Constipation | 52 | ♀ | 51 | 57 |
| Case 7 | Duodenal ulcer | 55 | ♀ | 37 | 70 |
| Case 8 | Arterial hypertension | 72 | ♀ | 61 | 49 |
| Case 9 | Arterial hypertension | 79 | ♂ | 43 | 99 |
| Case 10 | Colitis | 62 | ♀ | 49 | 68 |
| *Control group* | | | | | |
| Case 3 | Parathyroid adenoma | 62 | ♂ | 68 | 70 |
| Case 11 | Kidney stone | 60 | ♀ | 54 | 97 |
| Case 12 | Arteriosclerotic heart disease | 43 | ♀ | 63 | 63 |
| Case 13 | Medically treated hyper thyroidism | 67 | ♀ | 56 | 100 |

Table II *Data of calcium metabolism*

| | Bone formation rate mEq/(kg × 24 h) | Calcium turnover mEq/(kg × 24 h) | Exchangeable calcium mEq/kg | Serum alkaline phosphatase units |
|---|---|---|---|---|
| *Hypercalcaemic group* | | | | |
| Case 1 | 1 57 | 2 49 | 9 40 | 3 3 |
| Case 1 (i v) | — | — | — | 3 6 |
| Case 2 | 1 23 | 1 59 | 3 90 | 4 8 |
| Case 3 | 0 63 | 0 73 | 4 60 | 1 8 |
| Case 4 | 0 72 | 0 81 | 2 80 | 2 0 |
| Case 5 | 0 10 | 0 48 | 3 90 | 3 2 |
| *Normocalcaemic group* | | | | |
| Case 6 | 0 49 | 0 65 | 3 18 | 0 9 |
| Case 7 (i v) | — | — | — | 1 4 |
| Case 8 | 0 23 | 0 37 | 3 15 | 1 3 |
| Case 9 (i v) | 0 50 | 0 82 | 2 25 | 3 0 |
| Case 10 | 0 74 | 1 17 | 3 53 | 3 3 |
| *Control group* | | | | |
| Case 3 | — | — | — | 1 8 |
| Case 11 (i v) | — | — | — | 1 5 |
| Case 12 | — | — | — | 1 9 |
| Case 13 | — | — | — | 1 7 |

Table III *The effect of calcitonin on the calcium concentration in serum*

| | Mean basal calcium concentration (±S D) (mEq/l) | Minimum calcium concentration after injection (mEq/l) | Maximum change in calcium concentration (mEq/l) | Total hypocalcaemic response (mEq/l) × h |
|---|---|---|---|---|
| *Hypercalcaemic group* | | | | |
| Case 1 | 6 80 (±0 33) | 6 00 | −0 80 | −16 17 |
| Case 1 (i v) | 7 03 (±0 18) | 5 83 | −1 0 | −2 56 |
| Case 2 | 6 13 (±0 11) | 5 14 | −0 99 | −23 57 |
| Case 3 | 7 09 (±0 07) | 6 48 | −0 61 | −9 05 |
| Case 4 | 6 13 (±0 10) | 5 75 | −0 38 | −3 06 |
| Case 5 | 6 15 (±0 19) | 5 43 | −0 72 | −2 08 |
| Mean | 6 55 | 5 77 | −0 78 | −12 75 |
| *Normocalcaemic group* | | | | |
| Case 6 | 4 93 (±0 06) | 4 60 | −0 33 | −5 58 |
| Case 7 (i v) | 5 11 (±0 04) | 4 58 | −0 53 | −0 88 |
| Case 8 | 5 04 (±0 11) | 4 78 | −0 6 | −3 00 |
| Case 9 (i v) | 5 21 (±0 04) | 4 98 | −0 23 | −2 65 |
| Case 10 | 5 09 (±0 04) | 4 76 | −0 33 | −4 [illegible]0 |
| Mean | 5 08 | 4 74 | −0 34 | −3 [illegible]6 |
| *Control group* | | | | |
| Case 3 | 6 70 (±0 12) | 6 69 | −0 01 | |
| Case 11 (i v) | 4 91 (±0 07) | 4 81 | −0 10 | |
| Case 12 | 4 88 (±0 10) | 4 72 | −0 16 | |
| Case 13 | 4 90 (±0 11) | 4 79 | −0 11 | |
| Mean | 5 35 | 5 25 | −0 10[b] | |

Difference significant by Student's *t* test ($p < 0.01$)
[b] Difference not significant by Student's *t* test ($p > 0.05$)

Table IV *The effect of calcitonin on the magnesium concentration in serum*

| | Mean basal magnesium concentration (± S.D.) (mEq/l) | Minimum magnesium concentration after injection (mEq/l) | Maximum change in magnesium concentration (mEq/l) | Total hypomagnesiaemic response (mEq/l) × h |
|---|---|---|---|---|
| *Hypercalcaemic group* | | | | |
| Case 1 | 1.97 (±0.05) | 1.77 | −0.[illegible]0 | −1.86 |
| Case 1 (i.v.) | 2.01 (±0.07) | 1.79 | −0.[illegible]2 | −2.[illegible]0 |
| Case 2 | 1.48 (±0.04) | 1.27 | −0.21 | −1.22 |
| Case 3 | 1.63 (±0.03) | 1.52 | −0.11 | −1.27 |
| Case 4 | *1.96 (±0.06)* | *1.78* | −0.18 | −1.[illegible]2 |
| Case 5 | 2.09 (±0.07) | 1.89 | −0.[illegible]0 | −2.68 |
| Mean | 1.86 | 1.67 | −0.19[a] | −1.74 |
| *Normocalcaemic group* | | | | |
| Case 6 | 2.08 (±0.10) | 1.79 | −0.[illegible]9 | 7.30 |
| Case 7 (i.v.) | 1.64 (±0.06) | 1.58 | −0.06 | |
| Case 8 | 1.68 (±0.04) | 1.54 | −0.14 | 1.51 |
| Case 9 (i.v.) | 1.61 (±0.04) | 1.60 | −0.01 | |
| Case 10 | 1.44 (±0.05) | 1.39 | −0.05 | |
| Mean | 1.69 | 1.58 | −0.11[b] | |
| *Control group* | | | | |
| Case 3 | 1.53 (±0.05) | 1.58 | +0.05 | |
| Case 11 (i.v.) | 1.75 (±0.06) | 1.77 | +0.02 | |
| Case 12 | 1.69 (±0.04) | 1.62 | −0.07 | |
| Case 13 | 1.67 (±0.04) | 1.68 | +0.01 | |
| Mean | 1.66 | 1.66 | 0.00[b] | |

Difference significant by Student's $t$ test ($p < 0.001$)
Difference not significant by Student's $t$ test ($p > 0.05$)

on the day preceding the injection. None of the patients showed signs of hypersensitivity to the preparation. The dose (45 MRC units) was given intramuscularly except in three cases (Tables III and IV) where it was injected intravenously. Four control patients received injections of the calcitonin vehicle and were studied in exactly the same way as the calcitonin group. The patients were fasting overnight until 1 p.m. on the day of injection. They had distilled water ad lib.

### *Experimental methods*

Blood was drawn from a cubital vein after a minimum of forearm stasis and the serum concentrations of calcium, magnesium, phosphate, sodium, potassium, alkaline phosphatase, creatinine and total protein were determined. At least five blood samples were taken before the injection, namely at 8 and 12 a.m., 4 and 8 p.m. on the day before and at 8 a.m. just before the injection. The mean basal values were calculated from these five blood samples. After the injection, blood samples were drawn at least once an hour as long as the hypocalcaemic effect could be registered.

Blood samples for determination of ionized, complexed, and proteinbound calcium were drawn before the injection and when the calcitonin effect was thought to be maximal.

Urine samples were collected without a catheter every four hours for twenty-four hours before the injection, and as long as blood samples were taken. The urinary excretions of calcium, magnesium, phosphate, sodium, and potassium were expressed per mg excreted creatinine in order to correct for errors in the urine collections.

When the basal serum calcium level had been re-established, the magnitude of the exchangeable calcium pool, the calcium turnover rate, and the bone formation rate were determined in all patients in the hypercalcaemic group and in four patients with normocalcaemia.

### *Analytical methods*

Calcium in serum, urine, and ultrafiltrate from serum was determined by the EDTA murexide method ([illegible]1). The mean normal serum value ± S.D. was [illegible].31 ± 0.17 mEq/l (coefficient of variation [illegible].6%). Ionized calcium was measured by a modification of the method of Rose (17) (coefficient of variation 0.9%). The complexed fraction was determined as a difference between non-protein-bound calcium (from the ultrafiltrate) and ionized calcium.

The technique described by Heany and Whedon (11) was used for the determination of the calcium turnover. Thirty to forty μCi $^{47}CaCl_2$ with a specific activity of [illegible]0 mCi per g calcium were injected intravenously and the specific radioactivity was determined in the serum

Table V *The effect of calcitonin on the phosphate concentration in serum*

| | Mean basal phosphate concentration (±S D) (mg/100 ml) | Minimum phosphate concentration (mg/100 ml) | Maximum change in phosphate concentration (mg/100 ml) |
|---|---|---|---|
| *Hypercalcaemic group* | | | |
| Case 1 | 2.84 (±0 14) | 2 04 | −0 81 |
| Case 1 (i v) | 3 19 (±0 65) | 2 54 | −0 65 |
| Case 2 | 3 96 (±0 38) | 2 97 | −1 00 |
| Case 3 | 2 20 (±0 21) | 1 36 | −0 84 |
| Case 4 | 2 26 (±0 24) | 1 73 | −0 53 |
| Case 5 | 2 99 (±0 41) | 1 88 | −1 11 |
| Mean | 2 91 | 2 08 | −0 82 |
| *Normocalcaemic group* | | | |
| Case 6 | 3 23 (±0 48) | 2 28 | −0 95 |
| Case 7 (i v) | 3 74 (±0 47) | 3 04 | −0 70 |
| Case 8 | 2 85 (±0 16) | 2 04 | −0 81 |
| Case 9 (i v) | 3 35 (±0 40) | 2 54 | −0 81 |
| | 4 06 (±0 42) | 2 94 | −1 11 |
| Mean | 3 44 | 2.56 | −0 87 |
| *Control g oup* | | | |
| Case 3 | 1 94 (±0 12) | 1 64 | −0 30 |
| Case 11 (i v) | 4 27 (±0 40) | 2 98 | −1 28 |
| Case 12 | 4 03 (±0 63) | 3 06 | −0 96 |
| Case 13 | 3 66 (±0 13) | 3 08 | −0 58 |
| Mean | 3 47 | 2 69 | −0 78 |

[a] Difference significant by Student s *t* test ($p < 0\ 001$)

three and five hours after the injection and daily for ten days From the decay curves in serum the total exchangeable calcium pool (mEq/kg) and the amount of calcium leaving the exchangeable pool per twenty four hours (calcium turnover) were estimated The contents of Ca in urine and faeces were determined daily during the ten days, and the bone formation rate was calculated from the calcium turnover and the amount excreted In eleven normal adults the following values ±S D were found total exchangeable calcium pool 3 0±0 7 mEq/kg

calcium turnover $0\ 67 \pm 0\ 16\ \frac{\text{mEq}}{\text{kg} \times 24\ \text{h}}$

and bone formation rate $0\ 61 \pm 0\ 12\ \frac{\text{mEq}}{\text{kg} \times 24\ \text{h}}$ (8)

Throughout the investigation the subjects were in bed and kept on a regular hospital diet.

Magnesium was determined by atomic absorpt on spectrophotometry (Perkin Elmer Atomic Absorption Spectrophotometer 290) using 1 % lanthanum oxide as a diluent. The mean normal value ±S D of sera from 23 normal adults was 1 72±0 06 mEq/l (coefficient of variation 1.0%) (16)

Phosphate was determined by a modification of the method descr bed by Fiske and Subbarow (6) The mean normal serum value as inorganic phosphorus ±S.D was 3.52±0 58 mg/100 ml (coefficient of variation 3 0%) Potass um and sodium were measured by emission flame photometry (coeff cient of variation 1 8 and 1 2% respectively) Alkaline phosphatase was determined by dephosphorylation of *p*-nitrophenyl phosphate (3) The mean normal serum value ±S D was 2 0±0 6 units (coefficient of variation 6%) Protein was determined refractometrically (coefficient of variation 3 5 ) and creatinine with a Technicon autoanalyzer (coefficient of variation 3 6 )

## RESULTS

The effects of calcitonin injection on serum calcium magnesium and phosphate concentrations are shown in Tables III–V Tables III and IV give the total responses as measured by planimetry of the areas below the mean basal lines in diagrams similar to Fig. 2 This was done only in cases where the maximum response was more than twice the standard deviation of the height of the base line

There was a significant reduction of the serum calcium concentration in both the hypercalcaemic and the normocalcaemic groups most pronounced in the hypercalcaemic group (average decreases 11 9 and 6 7 respetively) The total hypocalcaemic effect ([mEq/l] × h) was however better correlated to (*a*) the bone formation rate (*b*) the

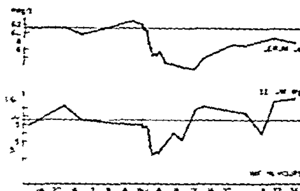


Fig. . The e ect of calcitonin injection on serum calcium and magnesium levels in a patient with hypercalcaemia (case .).

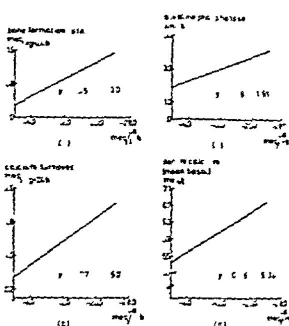


Fig 3 Correlation coefficients, r between the total hypocalcaemic response and (a) bone formation rate, r = 0.82, 0.01 > p > 0.001 (b) calcium turnover r = 0 4 0.05 > p > 0.02, (c) alkaline phosphatase level, r = 0.0 0.05 > p > 0.02, (d) basal serum calcium, r = 0.64 0.05 > p > 0.02.

calcium turnover and (c) the alkaline phosphatase level, than to (d) the basal serum calcium concentration (Fig. 3) Less clear correlations were found when the maximum hypocalcaemic response (mEq l) was used. No correlation to the total exchangeable calcium pool could be demonstrated (correlation coefficient 0.54 $p > 0.1$)

No changes were observed in the alkaline phosphatase levels after the injections.

Fractionated serum calcium de erminations were de in seven cases. A total average fall of 0 37 ... l was found. Thirty-eight % of this fall took place in the protein-bound, forty-six % in the ionized, and sixteen % in the complexed fraction. The resulting distribution does not differ significantly from that found befo e the injection.

There was a significant hypomagnesaemic response in the patients of the hypercalcaemic group (Table IV) In this group the mean percentage decrease of serum magnesium was of the same order of magnitude as that of calcium (10.2 and 11.9% respectively) Except in two cases (nos. 6 and 8) no considerable reduction in serum magnesium was observed in the normocalcaemic group The serum calcium and magnesium concentrations decreased in parallel (Fig. 2) Four of the six patients showing a lowering of serum magnesium had an elevated mean basal magnesium concentration.

The hormone effects on serum calcium and magnesium were not difficult to measure because of the relatively stable base lines (Tables III and IV) Significant decreases in serum phosphate we e seen in both the hypercalcaemic and normocalcaemic groups. The minimum phosphate concentrations after calcitonin injection (Table V) were

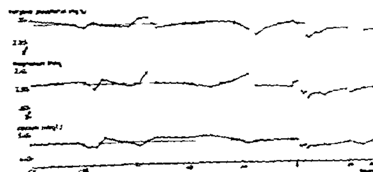


Fig 4 Variations of serum ... cal cium and magnesium during ... days showing effect of calcitonin in case 6 Arrow indicates time of injection

registered within twenty hours after the injection In five cases however lower concentrations due to spontaneous fluctuations were seen either on the day before or on the day after the injection Furthermore the controls also showed a significant fall in serum phosphate (Table V) As a consequence we could not estimate a possible effect on the serum phosphate The spontaneous fluctuations in serum phosphate magnesium and calcium of one patient (case 6) are shown in Fig 4

No changes were observed in the serum concentrations of protein potassium or sodium in any of the patients

We registered neither an immediate nor a prolonged effect of calcitonin on the urinary excretion of calcium magnesium phosphate or potassium. However the excretion rate of sodium was increased by more than 20% in seven out of nine patients during the first four hours after calcitonin injection A similar increase was seen in one of the four patients receiving a control injection (Table VI)

Table VI *Urinary excretion pattern following calcitonin injection*

Urine collected during the first four hours after injection compared with a similar sample collected 24 hours before

| | | No of patients with | | |
|---|---|---|---|---|
| | | >20 increase | >20 decrease | <20 increase or decrease |
| Ca | Hypercalcaemic | 2 | 1 | 1 |
| | Normocalcaemic | 2 | 0 | 3 |
| | Control | 0 | 3 | 1 |
| Mg | Hypercalcaemic | 0 | 1 | 3 |
| | Normocalcaemic | 2 | 2 | 1 |
| | Control | 0 | 1 | 3 |
| P | Hypercalcaemic | 1 | 1 | 2 |
| | Normocalcaemic | 4 | 0 | 1 |
| | Control | 1 | 1 | 2 |
| K | Hypercalcaemic | 1 | 1 | 2 |
| | Normocalcaemic | 3 | 0 | 2 |
| | Control | 1 | 0 | 3 |
| Na | Hypercalcaemic | 3 | 0 | 1 |
| | Normocalcaemic | 4 | 0 | 1 |
| | Control | 1 | 1 | 2 |

[a] Four only since urine samples were missing from cases 2 and 1 (i m)

## DISCUSSION

Each of our patients received a dose of 45 MRC units and showed a hypocalcaemic response of the same size as that found by Bijvoet et al (4) who used only 6 MRC units per patient in their study Haas and Dambacher (9) claimed that a maximum calcitonin effect was obtained with as little as 2 MRC units Later however they stated that inconsistent results are obtained with doses less than 10 MRC units (10)

The present work stresses the importance of the use of long base lines for the evaluation of the calcitonin effect on serum calcium magnesium and phosphate Kyner and Meck (13) found significant variations in the serum calcium level of a patient with hypercalcaemia secondary to malignancy and ascribed this to diurnal variations in the degree of hydration We have registered similar fluctuations in the two patients with multiple myeloma

In all the other patients of this material the diurnal variations in the serum calcium and magnesium levels were relatively small As pointed out by Carruthers et al (5) there are normally great fluctuations in the serum phosphate concentration The highest values occur in the afternoon and evening which is probably due to food intake This made it difficult for us to evaluate the calcitonin effect on serum phosphate

The greatest hypocalcaemic effect of calcitonin was seen in patients with hypercalcaemia The response was significantly correlated to the bone formation rate Provided that bone formation rate equals bone resorption rate this is what one might expect, as the hormone acts by inhibiting the bone resorption Our results are in accordance with the findings of Bijvoet et al (4) but are not quite in agreement with those of Haas and Dambacher (10) who found that the effect was well correlated to the degree of hypercalcaemia

In rat experiments it has been shown that porcine calcitonin has a hypomagnesiaemic effect in young animals which have a rapid bone turnover whereas no significant changes could be demonstrated in older animals (19) In our study a calcitonin induced hypomagnesiaemic effect was registered in the hypercalcaemic group in which on an average both the bone turnover and the basal serum magnesium level were elevated

The duration of the hormone effect varied from patient to patient and was most prolonged in the

patients with the highest bone turnover One patient received calcitonin first by the intramuscular and later by the intravenous route The hypocalcaemia lasted for thirty two and twenty eight hours respectively This seems to indicate that the duration is not a question of resorption In the hypercalcaemic patients the parathyroid hormone secretion rate would not be expected to rise after the calcitonin injection as the serum calcium of none of the patients decreased to values below 5 mEq/l This might be the explanation for the longer duration of the hypocalcaemic response in this group

We did not find any significant changes after calcitonin injection in the urinary excretion pattern apart from an increased sodium excretion rate This was also found by Singer et al (18) and by Bijvoet et al (4)

Other investigators have found controversial results Ardaillou et al (1) registered an increased renal excretion rate of calcium and phosphate The fact that an increased phosphate excretion was also found in two patients with hypoparathyroidism made them suggest that calcitonin inhibits the tubular reabsorption of phosphate since an increased compensatory parathyroid hormone secre could not be the explanation Bijvoet et al ) found a delayed decrease of the calcium ex ... on rate often preceded by a small increase Their findings seem however to be inconclusive because of similar variations in the controls In seven out of eleven patients they registered an elevated phosphate clearance after calcitonin injection They suggested that this might be due to an increased parathyroid hormone secretion Haas and Dambacher (9) were not able to show any significant changes in the clearance of calcium magnesium or phosphate whereas Singer et al (18) found augmented excretion rates of calcium magnesium phosphate and sodium after large doses of calcitonin

Our negative results concerning calcium magnesium and phosphate excretion may have several explanations 1) the urine collection periods have been too long to detect variations of short duration 2) the creatinine clearance was markedly reduced in one and slightly in five of the patients and 3) calcitonin has no effect on the renal excretion of the elements mentioned in the doses we have given

REFERENCES

1 Ardaillou, R., Vuagnat, P., Milhaud G & Richet, G Nephron 4 298 1967
2 Bell N H Barrett R J & Patterson, R Proc Soc. exp Biol. 123 114 1966
3 Bessey O A. Lowry O H. & Brock M J J biol Chem. 164 321 1946
4 Bijvoet O L. M., Veer van der Sluys, J & Jansen, A. P Lancet 1 876, 1968
5 Carruthers, B M Copp D H & MacIntosh H W J Lab clin. Med 63 959 1964
6 Fiske C. D & Subbarow Y J biol Chem. 66 375 1925
7 Foster G V Joplin, G F., MacIntyre I Melvin, K. E. W & Slack E. Lancet 1 107 1966
8 Friis, T & Weeke E Acta med scand. 187 41 1970
9 Haas, H G & Dambacher M Schweiz. med. Wschr 98 727 1968
10 — Lancet 2 217 1968
11 Heany R P & Whedon G D J clin Endocr 18 1248 1958
12 Kumar M A Slack E., Edwards, A Soliman, H A A Baghdiantz, A. Foster G V & MacIntyre I J Endocr 33 469 1965
13 Kyner J L & Meck, F C J clin Endocr 7 1755 1967
14 Lowry O H Rosebrough N Y Farr A. L. & Randall R. J J biol Chem 193 265 1951
15 Milhaud, G Moukhtar M S Bourichon, J & Perault, A. M C R Acad Sci (Paris) 61 4513 1965
16 Nielsen S P Scand J clin. Lab Invest. 1969 In press.
17 Rose G A Clin chim Acta 2 227 1957
18 Singer F R. Foster G V Joplin G I Nadarajah A Parkinson D K., Thalassinos, N Woodhouse N J Y., Clark M B., Fraser T R & MacIntyre I Calc Tiss. Res Suppl 20 1968
19 Soliman H A. A Studies in calcium and magnesium metabolism Thesis. University of London London 1966
20 Tenenhouse A Arnaud, C & Rasmussen H Proc. nat. Acad. Sci 53 818 1965
21 Wilkinson R H J clin Path 10 1 6 1957

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# SURVIVAL AND MORTALITY IN MALIGNANT (GRADE IV) AND GRADE III HYPERTENSION

*Trends in Consecutive Actively Treated Groups*

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*Abstract* A series of malignant and other severe hypertensives (grade IV 1 8 subjects grade III, 177 subjects, according to the classification of Keith, Wagener and Barker) were submitted to energetic antihypertensive treatment, starting in October 1950 The patients have been followed from 5 to 17 years Overall five year survival rates were in grade IV 50% in grade III 61% These results compare favorably with the results reported by other investigators

Subdividing the material into four consecutive patient groups as regards the start of treatment the most striking increase in five year survival in comparison with earlier untreated control material was observed already in the first group starting treatment in the early fifties The tendency to further improvement was rather slight However more advanced age and signs of more severe vascular damage in vital areas in the later patient groups might partly explain this patients without such unfavorable factors showing a better prognosis in the last two groups

Of the four major causes of death congestive failure was most markedly suppressed cerebro vascular lesions and uremia clearly lowered while myocardial infarction remained virtually unaffected

Measures discussed for further improving prognosis were active tracing and energetic treatment of less advanced stages of hypertensive disease improved detection of impaired renal function, prophylactic measures against pyelonephritis and an aggressive approach to simultaneous control of other coronary and cerebro vascular high risk factors as defined by prospective studies

Active medicamental treatment of hypertension albeit in a crude form became possible in 1950 In 1951–52 it was apparent that the malignant syndrome might be reversed and life considerably prolonged From the later half of the fifties a number of reports have appeared which showed 20–50 5 year survival of this disorder which earlier was regularly fatal within a 5 year period (Table I *a*)

We have earlier in 1956 1960 1961 1963 and 1966 published results of active treatment in malignant (grade IV) hypertension (4 5 12 13 14) *The five year survival rate had improved* strikingly We could not observe what Dustan et al (7) had labelled delayed uremia in the presence of satisfactory blood pressure control On the contrary we found that progress to lethal uremia only occurred either when renal impairment initially was severe (serum creatinine > 3 mg %) when blood pressure control was definitely deficient or when initially latent chronic pyelonephritis progressed

We have earlier also tried to analyze (13 14) whether there was a continuous trend of improvement in the results of treatment in the whole material of initially hospitalized hypertensives by fractionating the actively treated patient series (990 cases) into those who started treatment in the early middle or late third of the period 1950–59 *It was clear that most of the improvement* was apparent already in those starting treatment in the first third of the 1950s There was some suggestion however that this might be due to the fact that during the later part of the study contraindications for active treatment in the form of too far advanced vascular disease were no longer considered as valid as in the early years There was seen to be a profound shift in the relative importance of the four major causes of death congestive failure virtually disappearing cerebro-vascular lesions and uremia diminishing while

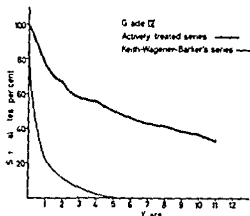



*Fig 1* Survival rates in grade IV hypertension of present actively treated material against the background of the Keith-Wagener-Barker series (17) Calculation of survival rates according to Berkson and Gage (3)

the rate of deaths due to myocardial infarction remained stable

The purpose of the present work was to see whether further improvement in results had appeared in more recent years in the most severe hypertensive syndromes or if not an attempt was to be made to analyze the reasons for stagnation in improvement

## MATERIAL

rom Oct 1 1950 to Dec. 31 1962, 1360 hospitalized patients below the age of 66 years were started on active antihypertensive treatment in the First Medical Department of Sahlgren's Hospital Goteborg, and the Medical Department of the Academic Hospital Uppsala. Of this material 1_8 had grade IV and 177 grade III fundi according to the Keith-Wagener-Barker classification (17)

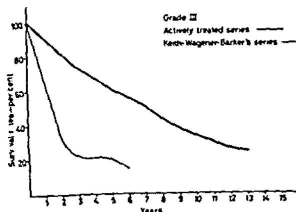



*Fig 2* Survival rates in grade III hypertension of present actively treated material against the background of the Keith-Wagener Barker series (17) Calculation of survival rates as given in Fig. 1

Table I*a* *Survival in malignant (grade IV) hypertension by type of treatment as reported by various authors*

| Authors | Year of report | Type of treatment | Per cent survival 4 y | 5 y | 6 y | 11 y |
|---|---|---|---|---|---|---|
| Keith et al (17) | 1939 | None | 2 | 1 | — | — |
| Page (21) | 1939 | None | 0 | 0 | 0 | — |
| Kincaid Smith et al (16) | 1959 | None | 3[a] | 1 | — | — |
| Bjork et al (4) | 1960 | None | 6 | 1 | — | — |
| Hodge et al (11) | 1961 | None | 0 | 0 | 0 | — |
| Palmer and Muench (2_) | 1953 | Diet | _0[a] | — | — | — |
| Peet and Isberg (?3) | 1948 | Sympathectomy | — | _2 | 18 | — |
| Björk et al (4) | 1960 | Sympathectomy | 15 | 15 | 10 | — |
| Barnett (1) | 1956 | Medical | _6 | — | — | — |
| Smirk ( 6) | 1956 | Medical | 56 | — | — | — |
| McMichael (18) | 1956 | Medical | — | 50 | — | — |
| Dustan et al (7) | 1958 | Medical | 48[a] | 33 | 6 | — |
| Perry and Schroeder (?4) | 1958 | Medical | 50 | — | — | — |
| Harington et al. (9) | 1959 | Medical | 25 | _2[a] | 18 | — |
| Bjork et al (4) | 1960 | Medical | 52 | 50 | 50 | — |
| Sokolow and Perloff (27) | 1960 | Medical | 15 | 15 | 8 | — |
| Mohler and Freis (19) | 1960 | Medical | _8[a] | 22 | — | — |
| Hodge et al (11) | 1961 | Medical | 46[a] | 36 | 33[a] | — |
| North et al (_0) | 1961 | Medical | 45 | 44 | — | — |
| Farmer et al (8) | 1963 | Medical | 37 | _9 | 3 | — |
| Harington M (10) | 1964 | Medical | — | 2_[a] | 0[a] | — |
| Present material | | | | | | |
| Series I 1950–56 | | Medical | — | 44 | 41 | ?7 |
| Series II 1957–62 | | Medical | — | 57 | — | — |
| Series I + II 1950–62 | | | — | 50 | | — |

[a] Estimate from survival curves.

Borderline cases with slight papilledema as well as unilateral papilledema were registered as grade III if other eye ground findings were consistent with this. Patients with papilledema, exudates and hemorrhages only observed during an attack of cerebral edema or cerebrovascular lesions were excluded if these signs were not registered also before or after the attack. Patients with tuberculosis of the kidney infected and contracted stone kidneys, polycystic kidney diabetes and chronic glomerulonephritis were excluded Patients with hypoplasia of the kidney as well as those with latent pyelonephritis with hypertension as the dominating symptom and first discovered during the diagnostic work up for hypertension or at autopsy were included. Patients with a primary diagnosis of chronic pyelonephritis, in whom hypertension was mild or moderate at the onset and who developed grade III or grade IV fundi in their subterminal phase were excluded. With isolated exceptions the diagnosis renal artery stenosis was not made in the two hospitals and operation not performed before 1960 On the strength

Table I*b* *Survival in grade III hypertension by type of treatment as reported by various authors*

| Authors | Year of report | Type of treatment | No of pats. ♂ | No of pats. ♀ | Per cent survival 5 y ♂ | 5 y ♀ | 6 y ♂ | 6 y ♀ | 11 y ♂ | 11 y ♀ |
|---|---|---|---|---|---|---|---|---|---|---|
| Keith et al (17) | 1939 | None | 22 | 15 | 9 | 7 | — | — | — | — |
| Hodge et al (11) | 1961 | Medical | 25 | | 10[a] | 33 | — | — | — | — |
| Breslin et al (6) | 1966 | Medical | 95 | 39 | 32 | 46 | — | — | — | — |
| Simpson and Gilchrist (25) | 1958 | Medical | 31 | 23 | 6 | 6 | — | — | — | — |
| North et al (20) | 1961 | Medical | 71 | | 57 | | | | | |
| Hodge et al (11) | 1961 | Medical | 119 | | 43[a] | 60[a] | — | — | — | — |
| Farmer et al (8) | 1963 | Medical | 68 | 28 | 32 | 54 | — | — | — | — |
| Present material | | Medical | | | | | | | | |
| Series I 1950–1956 | | | 37 | 64 | 48 | 64 | 48 | 63 | 16 | 44 |
| Series II 1957–1962 | | | 41 | 35 | 66 | 63 | — | — | — | — |
| Series I+II (1950–62) | | | 78 | 99 | 58 | 64 | — | — | — | — |
| Series I+II ♂+♀ | | | 177 | | 61 | | | | | |

[a] Estimate from survival curves

of the argument that the series during earlier years must have included a number of patients with unrecognized renal artery stenosis, the non-operated patients diagnosed during 1960–1962 were included. These numbered six teen patients with fundi of grade IV and eleven with fundi of grade III. The operated group of renal artery stenosis (1960–6 ) consisted of fifteen patients of whom eight with fundi of grade IV and seven with fundi of grade III. These were excluded.

*Definition of active antihypertensive treatment*

The criteria for inclusion in the actively treated series were the following. It was considered that active treatment in essence should be a combination treatment with two or more drugs. Some exceptions from this rule were made. Treatment with hexamethonium alone was considered active during the first 7–8 months of 1950–51 when this drug was the only one available. Treatment with rauwolfia derivatives or saluretics used as single drugs was considered active if it led to diastolic blood pressures ⩽ 100 mm Hg, otherwise a second drug should have been tried in order to include the patients in the actively treated series.

The general basis of treatment was a combination of a neurogenic-blocking agent with hydralazine or after 1956 with a saluretic. In succession we used peroral or parenteral hexamethonium, peroral or parenteral pentholinium, then guanethidine and during the last five years bethanidine.

A combination of saluretic and neurogenic blocking agent and hydralazine was regularly used in malignant and other severe varieties. In milder hypertension or elderly patients combinations were used of saluretic + hydralazine, saluretic + alphamethyldopa, or alphamethyldopa + hydralazine.

To be considered to have entered active treatment the patients should, after leaving the hospital, have reappeared for one outpatient visit and not just simply vanished. The control of the patients after leaving the hospital was usually spread over a large number of hands, including the general practitioner or various hospitals at a distance whence the patient had originally been referred to us. However more than half of the grade IV and grade III patients, and particularly those with the most severe problems, for instance advanced renal insufficiency were handled by the hypertension team.

Patients who had no outpatient treatment because they were in a subterminal state when treatment was instituted and died in the hospital were included in the actively treated series.

## RESULTS

*Survival*

In Figs. 1 and 2 and Tables I and II survival for our series has been considered in the light of survival data reported by various authors. Figs. 1 and 2 present the survival curves of our total series of cases of grade IV and III respectively as compared to the survival curves of the series of untreated cases given by Keith et al (17). Tables I*a* and I*b* present the percentage survival for our whole series and comparable data of various authors reporting untreated or treated series. Table I*a* which gives data for malignant (grade IV) hypertension shows that the five and six year survival rates of treated cases are still rather low even in the series published in recent years. This appears surprising at least viewed against the background of our increasing personal experience of well-controlled patients with malig

Table II *Causes of death in 201 cases of grade IV and grade III hypertension submitted to active antihypertensive treatment*

Total treated material 305 subjects (grade IV 128 grade III 177)

| | Grade IV | Grade III |
|---|---|---|
| *Deaths due to the four major causes* | | |
| Cerebrovascular lesions | 24 | 43 |
| Myocardial infarction | 11 | 34 |
| Congestive heart failure | 3 | 10 |
| Uremia | | |
| Chronic pyelonephritis | 10 | 4 |
| Other | 23 | 5 |
| *Deaths from other vascular and cardiac manifestations* | | |
| Dissecting aortic aneurysm | 1 | 2 |
| Pulmonary embolism | | 4 |
| Occluded mesenterial artery | | 1 |
| Valvular disease (mitral) | | 1 |
| *Deaths indirectly related to the hypertensive disease* | | |
| Pneumonia | 3 | 2 |
| *Deaths related to the regimen used* | | |
| Hexamethonium lung | 1 | |
| Suicide (reserpine) | 1 | |
| Paralytic ileus (Mevasine) | | 1 |
| edema | | 1 |
| *Deaths with no relation to the hypertensive disease* | | |
| Tumors | 1 | 9 |
| Various causes sepsis, pancreatitis, ulcerative colitis | 2 | 3 |
| *Incomplete records* | | 1 |
| Total | 80 | 121 |

nant hypertension in good or excellent shape after observation periods from 10 to 17 years The five year survival rate for our total series 1950–1962 was 50 %. Table I b gives data for grade III cases The five year survival rate for our total series of this grade was 61 % In both grade IV and grade III there was some improvement in the 1956–1962 series as compared to the 1950–1956 series As expected the prognosis was better for females than for males

*Causes of death*

Table II shows the causes of death in our entire series. In the series of 128 grade IV patients 60

Table III *Type of lethal cerebral vascular lesions Autopsy findings*

| | No autopsy performed | Cerebral hemorrhage | Cerebral infarction | Total |
|---|---|---|---|---|
| Grade IV | 11 | 8 | 5 | 4 |
| Grade III | 10 | 23 | 10 | 43 |

died and in the series of 177 grade III patients 121 died As expected uremia played a far greater role as cause of death in grade IV than in grade III hypertension In both grade IV and grade III about one third of the deaths were caused by cerebral vascular lesions The proportion of deaths of myocardial infarction was greater in grade III than in grade IV hypertension As is usually the case in treated hypertension congestive failure was the cause of death only in a small number of patients Five deaths of pneumonia have been listed as deaths indirectly related to the hypertensive disease We have done this because we think that with to-day's therapeutic possibilities these deaths would not have occurred if there had been no underlying circulatory disturbance present

Table III gives a breakdown of lethal cerebral vascular lesions into cerebral hemorrhage and cerebral infarction in the part of the series in which an autopsy was performed No definite differences in the proportions for the two grades were seen

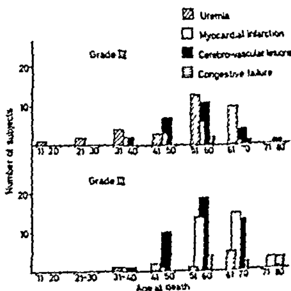



*Fig 3* The age at death from the four major causes in actively treated grade IV and grade III hypertension




*Fig 4* Five year survival and mortality in per cent in relation to the period when active antihypertensive treatment was started. Background material the untreated series of Keith-Wagener-Barker (17) □ alive >5 years ▨ dead within 5 years

Fig. 3 presents the age distribution of the four major causes of death As would be expected uremia dominated the early age deaths in grade IV In both grades cerebrovascular lesions began to be of importance in the fifth decade. Congestive failure appeared almost solely in the later decades of grade III

*Consecutive trends in mortality*

The first powerful agent used in this series hexamethonium perorally or parenterally was a harsh drug to use particularly as a single agent. From the end of 1951 hexamethonium was given in combination with hydralazine and later on new drugs were used as they became available The drop-outs from treatment during the days of hexamethonium as a single agent were very frequent. It might reasonably be expected that, with better drugs and increasing experience results should show a steady tendency to improve

In order to study this we have subdivided our total series into four periods as regards the start of treatment. Period I comprises patients starting treatment Oct. 1 1950 – Dec. 31 1953 period II 1954–1956 period III 1957–1959 and period IV 1960–1962. Follow up observation terminated on Dec. 31 1967 thus giving at least 5 years observation. In a number of our tables these periods have been pooled into the first series" (1950–1956) and the second series" (1957–1962)

In Fig. 4 five year survival and mortality in cases starting treatment in the four periods are

Table IV *The four major causes of death within 5 years in grade IV and grade III hypertension for patients starting treatment in four consecutive periods*

| | First series 1950–1956 | | | | Second series 1957–1962 | | | |
|---|---|---|---|---|---|---|---|---|
| | Period I $n=33$ | Period II $n=37$ | Period I+II $n=70$ | (%)[a] | Period III $n=32$ | Period IV $n=$[illegible]6 | Period III+IV $n=58$ | (%)[a] |
| *Grade IV* | | | | | | | | |
| Cerebrovascular lesions | 9 | 4 | 13 | 19 | 4 | 1 | 5 | 9 |
| Myocardial infarction | 2 | 4 | 6 | 9 | — | 2 | 2 | 3 |
| Uremia | 11 | 6 | 17 | 24 | 6 | 8 | 14 | 24 |
| Congestive heart failure | — | 1 | 1 | 1.5 | 1 | — | 1 | 2 |
| | Period I $n=29$ | Period II $n=72$ | Period I+II $n=101$ | | Period III $n=48$ | Period IV $n=$[illegible]8 | Period III+IV $n=76$ | |
| *Grade III* | | | | | | | | |
| Cerebrovascular lesions | 4 | 18 | 22 | 21 | 5 | 3 | 8 | 10 |
| Myocardial infarction | 1 | 8 | 9 | 9 | 5 | 4 | 9 | 12 |
| Uremia | — | 1 | 1 | 1 | 3 | — | 3 | 4 |
| Congestive heart failure | 3 | 1 | 4 | 4 | — | 1 | 1 | 1 |

$n$ = Total number of individuals starting treatment in each period
[a] % of total number of individuals starting treatment in the periods.

Table V *Distribution of age at start of treatment in grade IV and grade III hypertension*

Frequency in each age group given as percentage of total number starting treatment in 1950–1956 (first series) and 1957–62 (second series) respectively

| | Grade IV | | Grade III | |
|---|---|---|---|---|
| Age | First series Period I+II 1950–1956 n=70 ( ) | Second series Period III+IV 1957–1962 n=58 ( ) | First series Period I+II 1950–1956 n=101 ( ) | Second series Period III+IV 1957–1962 n=76 ( ) |
| <40 | 16 | 12 | 8 | 4 |
| 41–50 | 33 | 31 | 25 | 24 |
| 51–60 | 44 | 41 | 54 | 46 |
| 61–65 | 7 | 16 | 14 | 26 |

presented against the background of survival of untreated cases as reported by Keith et al (17) There was a sharp increase in five year survival for the patients starting treatment in the first years of the fifties In the periods thereafter there is seen to be a somewhat stagnating trend in further improvement not least marked in patients starting treatment 1960–1962

Table IV tries to answer the question whether has occurred a shift with time in the relative ice of the four major causes of death in hypertension The impressive feature was a drop in the number of lethal cerebral vascular lesions the percentage being halved in grade IV as well as in grade III in the second 6 year period as compared to the first 6-year period As regards other causes of death no certain difference was seen

### *Analysis of possible reasons for stagnation in improvement of 5 year survival*

As stated above the general impression is that antihypertensive treatment has successively become more flexible with more alternative forms and thereby easier to maintain In spite of this we have found very little successive improvement in 5 year survival in patients starting treatment in consecutive periods However this finding might in reality be erroneous and explainable by widening indications for active treatment into groups of patients of older age and with more advanced vascular disease. In order to answer this question we have analyzed separately the distribution of age the frequency of severe signs from brain heart and kidneys at the start of treatment as well as the degree of control or escape from control for patients starting treatment in different periods of time We present the results in Tables V–VII

Table V presents the series divided into four age groups. In each age group the frequency has been given in percentage of total number starting treatment in the two consecutive 6-year periods 1950–56 and 1957–62.

Table VI gives the frequency of signs of vascular damage of heart, brain or kidneys at the beginning of treatment for patients starting in the two consecutive periods, as well as the number of deaths in the various groups of patients.

Table VII gives the degree of blood pressure control or escape from control of patients starting in the two 6-year periods as well as the relation of this to mortality

From Table V it is evident that from the first to the second 6 year period of the study the proportion of subjects above 60 years of age increased in both grades IV and III while the opposite occurred for subjects below the age of 40 years

Table VI shows no higher proportion of patients with heart disease or brain injury in the later as compared to the earlier 6-year period However the records of the cases of the first years more often showed general cerebral deterioration and focal cerebral injury than strokes without or with minor sequelae The proportion of patients with renal impairment at the start was much greater in the later than in the earlier 6-year period The difference would not have been significantly smaller if we had included in the series the 16 patients operated upon for renal artery stenosis none of whom had a serum creatinine above 2 mg all belonged to the later 6-year period

Table VII shows that the number of patients achieving first class control did not appear to have increased much in the second part of the study but total escapes from control appeared to have become definitely rare The many escapes from treatment during the first years may be explained by a number of factors firstly by the high frequency of patients with general cerebral deterioration secondly by the difficulties during the first year with hexamethonium as the only drug and thirdly by the lack of experience on the part of the physicians. It should be noted that with increasing experience a slow steady increase of drugs with ensuing slow reduction of blood pressure has effected a number of striking reversals of what initially appeared as irreversible

Table VI *Mortality within 5 years in relation to signs from heart brain and kidney at start of treatment Two consecutive series*

| | Grade IV | | | | | | Grade III | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | First series Period I+II 1950–1956 | | Second series Period III+IV 1957–1962 | | Total | | First series Period I+II 1950–1956 | | Second series Period III+IV 1957–1962 | | Total | |
| | *n* 70 | D 39 | *n* 58 | D 25 | *n* 128 | D 64 | *n* 101 | D 42 | *n* 76 | D 27 | *n* 177 | D 69 |
| Coronary heart disease | 5 | 1 | 3 | 2 | 8 | 4 | 28 | 10 | 19 | 8 | 47 | 18 |
| Congestive failure | 14 | 11 | 9 | 6 | 23 | 17 | 18 | 13 | 8 | 6 | 26 | 19 |
| Cerebrovascular lesions and/or cerebral deterioration | 29 | 18 | 16 | 5 | 45 | 23 | 34 | 19 | 35 | 11 | 69 | 30 |
| Renal insufficiency (*a*) Serum creat >2 mg or NPN >40 mg | 13 | 8 | 24 | 11 | 37 | 19 | 17 | 9 | 16 | 4 | 33 | 13 |
| (*b*) Serum creat >4 mg or NPN> 60 mg | 4 | 3 | 7 | 6 | 11 | 9 | 1 | 1 | 3 | 3 | 4 | 4 |
| Signs from two or more regions | 15 | 12 | 12 | 8 | 27 | 20 | 22 | 14 | 16 | 10 | 38 | 24 |
| No signs | 23 | 10 | 23 | 4 | 46 | 14 | 33 | 6 | 23 | 5 | 56 | 11 |
| Diast BP >150 | 56 | 35 | 41 | 16 | 97 | 51 | 43 | 22 | 29 | 10 | 72 | 32 |

*n* = Number of treated patients D = Number of dead within 5 years

mental deterioration in grade IV hypertension On the strength of an earlier analysis Hood et al (14) we would assume that the better maintenance of blood pressure control is a reasonable explanation of the reduction of lethal cerebrovascular lesions in the later years

The general impression from this analysis would be that treatment seems to have improved somewhat mainly by total escapes becoming more rare The failure to decisively improve 5 year survival seems at least partly explainable by a somewhat higher age and more frequent kidney impairment at the start of treatment in later periods

These facts as well as the earlier demonstration (14) that the deaths from myocardial infarction in more than half of the cases occurred in well or excellently controlled patients are clear indications of the limitations of the effect of treatment to be expected in this type of patients even if serious attempts at blood pressure control have been made It is a truism that myocardial infarction is a common occurrence in subjects who are and always have been completely normotensive

Table VII *Mortality within 5 years in relation to degree of blood pressure Two consecutive series*

| | Grade IV | | | | | | Grade III | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | First series Period I+II *1950–1956* | | Second series Period III+IV *1957–1962* | | Total | | First series Period I+II *1950–1956* | | Second series Period III+IV *1957–1962* | | Total | |
| Degree of control / Lack of control | *n* 70 | D 39 | *n* 58 | D 25 | *n* 128 | D 64 | *n* 101 | D 42 | *n* 76 | D 27 | *n* 177 | D 69 |
| BP <100 mm Hg | 13 | 3 | 17 | 3 | 30 | 6 | 14 | 1 | 16 | 2 | 30 | 3 |
| Decrease >20 mm Hg | 28 | 13 | 25 | 12 | 53 | 25 | 35 | 17 | 23 | 8 | 58 | 25 |
| Decrease <20 mm Hg | 5 | 4 | 6 | 6 | 11 | 10 | 15 | 9 | 14 | 10 | 9 | 19 |
| Still on drugs Effect unknown | 2 | — | 6 | 1 | 8 | 1 | 14 | — | 7 | 1 | 21 | 1 |
| Drugs abandoned | 22 | 19 | 4 | 3 | 26 | 22 | 23 | 15 | 16 | 6 | 39 | 21 |

*n* = Number of treated patients D = Number of dead within 5 years

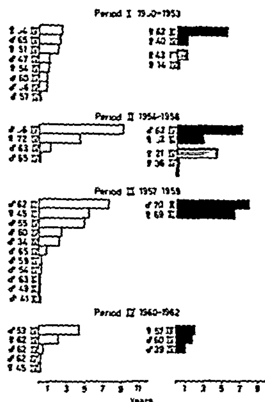



*Fig 5* Sex, age at death, eyegrounds and length of survival in patients dying of uremia who started treatment in different periods. III and IV grade according to Keith-Wagener-Barker. ☐ arteriolar sclerosis and arteriolar rosis, ☐ chronic pyelonephritis, ☰ malformation of kidney

The essential independence of the atherosclerotic process is a fact even if it is accelerated by hypertension

Subjects dying of uremia have been subjected to a detailed analysis. The results are presented in Fig. 5 where diagnosis and survival times are given for patients starting treatment during different periods. All cases were autopsied.

One may raise the question whether more cases of latent pyelonephritis might be included in the series of the later periods and thus by blood pressure independent progress might make decrease of mortality by uremia more difficult to achieve. There was no evidence however of pyelonephritis becoming more important in the later series. It may be noted that in the periods 1957–1962 there appeared a number of very short lived grade IV patients with essential hypertension in the malignant phase and with autopsy findings of severe arteriolar sclerosis as the dominating feature. These as a rule represented long-distance referrals who were sent in by their physicians as a desperate measure with a serum creatinine level of 4–10 mg%. This obviously may be one of the contributory reasons for our failure to improve results.

As regards hypoplasia (or dysplasia) of the kidney it seems appropriate to point out that according to our whole collected experience (76 subjects 1950–1967 representing all grades) these subjects were on the whole easy to control. Of those dying in the early periods three arrived in a subterminal stage and one escaped from excellent control after more than four years due to a series of unfortunate circumstances.

*Evaluation of the relations between initial injury in vital areas and subsequent degree of B.P control. Causes of death in relation to the control of B.P*

The analysis comprises the whole series of 128 cases of grade IV and 177 cases of grade III hypertension

Tables VIII *a* and *b* present the blood pressure control achieved in patients with signs of vascular damage in various vital circulatory areas or without any such signs as well as the control achieved in patients with initially extremely high diastolic pressure levels. Also noted are the number of deaths in each group of cases which occurred during the whole observation period (17.5 years).

Coronary heart disease and congestive failure have been listed separately. If both were present together the subject was referred to the one of the two diagnoses which appeared dominating. Patients with signs from the brain have been placed in one group although it was realized that there is a vast difference of importance between a single attack leaving no permanent damage, on the one hand, and the combination of repeated strokes with pronounced mental deterioration and possibly severe focal sequelae on the other. Non-protein nitrogen was in the first years of the study the sole criterion of renal function in the majority of cases. For several years it was then determined in parallel with serum creatinine whereafter it was dropped. During the period of determination of both parameters we found the limits NPN > 40 mg% and creatinine > 2 mg% for mild renal insufficiency and NPN > 60 mg% and creatinine > 4 mg% for severe renal impairment, to correspond well with each other.

Tables IX *a* and *b* give the number of deaths from various causes in relation to the blood pressure control achieved.

Table VIII shows that only about one third of the cases of both grade IV and III had no signs of injury from heart brain or kidneys at the start of treatment. As expected, the survival was far better in this category of cases than in the others

Table VIIIa *Degree of blood pressure control and escapes from treatment in relation to signs from heart brain and kidney at start of treatment*

| | Degree of control | | | | | | Lack of control | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Diastolic BP | | | | | | Still on drugs or on drugs at death | Effect unknown | Drugs abandoned | | Total | |
| | Normalized | | Decrease mm Hg >20 | | <20 | | | | | | | |
| Initial signs | *n* | D | *n* | D | *n* | D | *n* | D | *n* | D | *n* | D |
| *Grade IV* | | | | | | | | | | | | |
| *n*=128 | | | | | | | | | | | | |
| Coronary heart disease | — | — | 4 | 3 | 1 | — | 1 | 1 | 2 | 1 | 8 | 5 |
| Congestive failure | 4 | 2 | 7 | 6 | 6 | 6 | — | — | 7 | 7 | 24 | 21 |
| CVL and/or cerebral deterioration | 6 | — | 16 | 14 | 3 | 3 | 1 | 1 | 12 | 12 | 38 | 30 |
| Renal insufficiency | | | | | | | | | | | | |
| (*a*) Serum creat >2 mg or NPN >40 mg | 6 | 2 | 16 | 12 | 2 | 2 | 1 | — | 6 | 6 | 31 | 22 |
| (*b*) Serum creat >4 mg or NPN >60 mg | 2 | 1 | 3 | 3 | 4 | 4 | — | — | 2 | 2 | 11 | 10 |
| Signs from two or more regions | 3 | 1 | 11 | 10 | 5 | 5 | — | — | 7 | 7 | 26 | 23 |
| No signs | 15 | 5 | 20 | 6 | 1 | 1 | 5 | — | 6 | 6 | 47 | 18 |
| Diastolic BP ≥150 mm Hg | 21 | 8 | 38 | 22 | 9 | 9 | 6 | 1 | 20 | 20 | 94 | 60 |

*n*=Number of patients D=Number of dead

Table VIIIb *Degree of blood pressure control and escapes from treatment in relation to signs from heart brain and kidney at start of treatment*

| | Degree of control | | | | | | Lack of control | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Diastolic BP | | | | | | Still on drugs or on drugs at death | Effect unknown | Drugs abandoned | | Total | |
| | Normalized | | Decreased mm Hg >20 | | <20 | | | | | | | |
| Initial signs | *n* | D | *n* | D | *n* | D | *n* | D | *n* | D | *n* | D |
| *Grade III* | | | | | | | | | | | | |
| *n*=177 | | | | | | | | | | | | |
| Coronary heart disease | 9 | 7 | 14 | 12 | 8 | 6 | 4 | — | 8 | 8 | 43 | 33 |
| Congestive failure | 5 | 4 | 10 | 10 | 8 | 8 | — | — | 3 | 3 | 26 | 25 |
| CVL and/or cerebral deterioration | 6 | 2 | 19 | 15 | 11 | 11 | 4 | — | 20 | 18 | 60 | 46 |
| Renal insufficiency | | | | | | | | | | | | |
| (*a*) Serum creat >2 mg or NPN >40 mg | 3 | 2 | 17 | 14 | 3 | 3 | 3 | — | 4 | 3 | 30 | 22 |
| (*b*) Serum creat >4 mg or NPN >60 mg | 1 | 1 | 2 | 2 | — | — | — | — | 1 | 1 | 4 | 4 |
| Signs from two or more regions | 4 | 4 | 18 | 16 | 8 | 8 | 1 | — | 5 | 5 | 36 | 33 |
| No signs | 11 | 4 | 16 | 11 | 8 | 5 | 11 | 2 | 9 | 7 | 55 | 29 |
| Diastolic BP ≥150 mm Hg | 12 | 8 | 27 | 24 | 9 | 7 | 9 | — | 17 | 16 | 74 | 55 |

*n*=Number of patients D=Number of dead

Table IX a. *Causes of death in grade IV hypertension in relation to degree of BP control or escape from treatment*

| Causes of death | Degree of control | | | Lack of control | | |
|---|---|---|---|---|---|---|
| | Diastolic BP | | | | | |
| | Normalized | Decrease, mm Hg >20 | Decrease, mm Hg <20 | On drugs at death. Effect unknown | Drugs abandoned | Total |
| *Grade IV* | | | | | | |
| Cerebrovascular lesions | — | 12 | 2 | — | 10 | 24 |
| Myocardial infarction | 3 | 8 | — | — | — | 11 |
| Congestive failure | — | 2 | — | — | 1 | 3 |
| Uremia | 5 | 7 | 7 | — | 14 | 33 |
| Other vascular causes | — | 1 | — | — | — | 1 |
| Indirectly related to hypertensive disease | — | 2 | — | 1 | — | 3 |
| Related to regimen used | — | 1 | 1 | — | — | [illegible] |
| No relation to hypertensive disease | — | 1 | — | 1 | 1 | 3 |
| Incomplete records | — | — | — | — | — | — |
| Total | 8 | 34 | 10 | 2 | 26 | 80 |

this, however, was true only if treatment was maintained. In grade IV control was better and escape from treatment somewhat less than in grade III. As might be expected, escape from treatment was most frequent in cases with initial brain injury. Diastolic levels below 100 mm Hg were obtained more frequently in grade IV than in grade III hypertension, particularly in cases [illegible] initial diastolic levels >150 mm Hg. Mor[illegible] was extremely high in cases with initial [illegible] impairment, particularly when this was severe. As regards patients with congestive failure at the start of treatment, out of 26 cases of grade III all but one died and out of 24 cases of grade IV all but three died. However, as may be seen from Table VIII, the main cause of death during treatment was only rarely congestive failure. Among patients who abandoned treatment there were very few survivors.

In order to avoid misinterpretation of the findings presented in Tables IX *a* and *b* one has to take into account the following facts. It is a matter of course that initial severe renal impairment often is the reason of deficient blood pressure control and that there then is progress to lethal uremia. Also severe initial brain injury often pre-

Table IX b. *Causes of death in grade III hypertension in relation to degree of BP control or escape from treatment*

| Causes of death | Degree of control | | | Lack of control | | |
|---|---|---|---|---|---|---|
| | Diastolic BP | | | | | |
| | Normalized | Decrease, mm Hg >20 | Decrease, mm Hg <20 | On drugs at death. Effect unknown | Drugs abandoned | Total |
| *Grade III* | | | | | | |
| Cerebrovascular lesions | 2 | 15 | 11 | 1 | 14 | 43 |
| Myocardial infarction | 6 | 13 | [illegible] | 1 | 7 | [illegible] |
| Congestive failure | 1 | 3 | 2 | — | 4 | 10 |
| Uremia | 1 | 5 | — | — | 3 | 9 |
| Other vascular causes | 2 | 3 | 2 | — | 1 | 8 |
| Indirectly related to hypertensive disease | — | — | 1 | — | 1 | 2 |
| Related to the regimen used | 1 | 1 | — | — | — | [illegible] |
| No relation to hypertensive disease | 2 | 5 | 1 | — | 4 | 12 |
| Incomplete records | — | — | — | — | 1 | 1 |
| Total | 15 | 45 | 24 | 2 | 35 | 121 |

vents effective blood pressure control and it is obvious that in such cases new strokes or progress to uremia will occur On the whole however the findings give support to earlier observations that the most frequent cause of death in well or ex cellently controlled hypertension was myocardial infarction and that cerebrovascular lesions mainly occurred in patients escaping from control or hav ing rather poor control

## DISCUSSION

In spite of the successively better possibilities for treatment of hypertension there was a certain trend towards stagnation in improvement of five year survival results in our series of cases of severe hypertension This might be only partly explained by a change towards a higher age distribution and an increased number of patients with advanced renal insufficiency at the start of treatment in the second half of our series We seem to be up against a stone wall when it comes to advanced renal insufficiency and atherosclerotic complications a fact which could be easily fore seen

In order to improve results in severe forms of hypertension one cannot confine the treatment to lowering of the blood pressure It will be neces sary also to utilize the full information about other factors of pathogenetic importance for atherosclerosis and for other kinds of impairment of vital organs deriving from neighbouring fields of medicine and to discuss practical proposals to combat them Reports from large scale prospective studies on high risk factors for ischemic heart disease and cerebrovascular lesions and the ex periences of long term dietary and drug treat ment of hyperlipidemic disorders should certainly be taken into consideration by everyone treating hypertensives

The question might be raised whether energetic antihypertensive treatment in moderately severe hypertension will prevent the progress to grades IV and III and thus diminish the appearence of cases of these grades The finding of the Veterans Administration Cooperative Study Group on anti hypertensive agents (28) of the appearence of five grade III and two grade IV fundi after short ob servation periods in a randomized placebo-treated group of seventy men is striking This and other results in the study strongly supported more vig orous treatment in earlier stages of hypertensive disease

Prevention of severe progressive pyelonephritis leading to malignant hypertension seems partly possible Both an analysis of all deaths in uremia in ages ≤60 years in Goteborg during the last 16 years and the experience of admitted and non admitted candidates in the transplantation pro gram (with at present 109 transplanted patients) showed a heavy dominance of the abuse of anal getic drugs in cases who reached advanced stages or died of non obstructive pyelonephritis Since the free purchase of these drugs was abolished on Feb 1 1961 there have definitely been signs of an improving situation according to several meth ods of observation Frequent examinations includ ing urine cultivation and long term treatment with chemotherapeutic regimens should also certainly prove of importance

The extremely high frequency of hyp ruricemia in hypertensives with renal impairment and thus often in grade IV and grade III cases seems also of importance The hyperuricemia may be of approximately the same magnitude as seen in gout with renal impairment and is seen particularly in cases treated with saluretics

Altogether the need for extensive biochemically oriented study both in the initial diagnostic work up of hypertensive cases and then serially during treatment is pressing The full power of auto-matized laboratory procedures should be brought into the field

Measures against high risk factors should cer tainly be taken whenever practically possible However a particularly difficult proposition is what one might call the multiple high risk indi vidual a middle aged man with excessive ciga rette smoking some obesity hypertriglyceridemia hypercholesterolemia hyperuricemia and hyper tension which may be of a severity that requires treatment with a combination of two or three drugs In such a situation a full control of all risk factors might lead to an intolerably cumbersome and polypharmaceutical regimen

The hereditary occurrence of severe hyperten sion is a common phenomenon as also seen in the present series We have therefore for several years although not systematically enough exam ined siblings and children of patients with serious hypertension and if the need existed offered treatment This policy leads naturally to a con

siderably higher yield of positive findings than a random screening program of a population. The psychological readiness and motivation to accept life long treatment is also definitely much higher in these relatives of hypertensives than in cases found during a general health survey Plans are presently being made to systematize this policy of case finding on a hereditary basis which we think definitely more worth while than large general screening programs that might bring forth great numbers of psychologically unprepared patients who could hardly be efficiently coped with by our present resources

## REFERENCES


1 Barnett, A. J Treatment of severe arterial hypertension. Results from long term use of methonium compounds with and without the addition of reserpine Aust. Ann. Med. 5 274 1956

2. Bengtsson, U & Hood, B Abuse of phenacetin containing analgesics and renal damage In Progress in pyelonephritis (ed. E. H. Kass) F A. Davis Co Philadelphia 1965

3 Berkson, J & Gage R. P Calculation of survival rates for cancer Proc. Mayo Clin. 25 270 1950

4 Bjork, S., Sannerstedt, R., Angervall, G & Hood, B Treatment and prognosis in malignant hypertension Clinical follow up study of 93 patients on modern medical treatment. Acta med. scand. 166 175 1960

5 Bjork, S Sannerstedt, R. Falkheden, T & Hood, B The effect of active drug treatment in severe hypertensive dis ase An analysis of survival rates in 381 cases on combined treatment with various hypotensive agents. Acta med scand. 169 673 1961

6. Breslin, D J Gifford, R W., Jr & Fairbairn, J F Essential hypertension. A twenty year follow up study Circulation 33 87 1966

7 Dustan, H. P., Schneckloth R. E. Corcoran, A. C & Page I. H. The effectiveness of long term treatment of malignant hypertension. Circulation 18 644 1958

8 Farmer R. G., Gifford, R. W & Hines, E. A. Effect of medical treatment of severe hypertension. A follow up study of 161 patients with group 3 and group 4 hypertension. Arch. intern. Med. 12_ 164 1963

9 Harington, M., Kincaid Smith, P & McMichael, J Results of treatment in malignant hypertension. A 7 year experience in 94 cases. Brit. Med. J 2 969 1959

10 Harington, M. Malignant hypertension. Practitioner 193 35 1964

11 Hodge, J V., McQueen, E. G & Smirk, H. Results of hypotensive therapy in arterial hypertension based on experience with 497 patients treated and 156 controls observed for periods from 1 to 8 years. Brit. Med. J 1 1 1961

12. Hood, B Five and a half years experience of combinations of hypotensive drugs. Main present difficulties. In. Hypotensive drugs, a symposium, p 135 (ed. M Harington) Pergamon Press, London 1956.

13 *Hood, B., Bjork, S., Sannerstedt, R. & Angervall G* Analysis of mortality and survival in actively treated hypertensive disease Acta med. scand. 174 393 1963

14 Hood, B., Aurell, M Falkheden, T & Bjork, S Analysis of mortality and survival in actively treated hypertensive disease In. Antihypertensive therapy (Principles and practice) An international symposium (ed. F Gross) p 370 Springer Verlag, Berlin, Heidelberg, New York 1966

15 Hood, B Falkheden, T & Carlsson, M Trends and present pattern of mortality in chronic uremia. Acta med scand 181 561 1967

16 Kincaid Smith P McMichael J & Murphy E. A. The clinical course and pathology of hypertension with papilloedema (malignant hypertension) Quart. J Med 27 117 1958

17 Keith, N M., Wagener H. P & Barker N W. Some different types of essential hypertension Their course and prognosis. Amer J med Sci. 197 332, 1939

18 McMichael J Results of methonium treatment in malignant hypertension. Five years follow up In. Hypotensive drugs (ed. M Harington) p 105 Pergamon Press, London 1956

19 Mohler E. R. Jr & Freis, E. D Five years survival of patients with malignant hypertension treated with antihypertensive agents Amer heart. J 60 329 1960

20 North, J D K. Williams, J C P & Howie R. N Severe hypertension treated with ganglion blocking drugs in a general hospital Brit. Med. J 1 14 6 1961

21 Page L H. A clinical study of mal gnant hypertension. Ann. intern. Med. 12 978 1939

22. Palmer R. A. & Muench H Course and prognosis of essential hypertension Follow up of 453 patients 10 years after original series was closed. J Amer med. Ass. 153 1 1953

23 Peet, M M & Isberg, E. M The problem of malignant hypertension and its treatment by splanchnic resection. Ann. intern. Med. 28 755 1948

24 Perry H. N Jr & Schroeder H A. The effect of treatment on mortality rates in severe hypertension. A comparison of medical and surgical regimens. Arch. intern. Med 102 418 1958

25 Simpson F O & Gilchrist, A. R. Prognosis in untreated hypertensive vascular disease Scot. med. J 3 1 1958

26 Smirk, F H. Principles and details of hypotensive therapy In Hypotensive drugs (ed. M Harington) p 109 Pergamon Press, London 1956.

27 Sokolow M & Perloff, D Five years survival of consecutive patients with malignant hypertension treated with antihypertensive agents. Amer J cardiol. 6 858 1960

28 Veterans administration cooperative study group on antihypertensive agents. Effects of treatment on morbidity in hypertension. Results in patients with diastolic blood pressure averaging 115 through 129 mm Hg. J Amer med ass. 202 116 1967

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# THE OCCURRENCE OF TWO IgG EARLIER UNKNOWN IN JOINT FLUID AND SERUM FROM RHEUMATOID ARTHRITIS

PRELIMINARY REPORT

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Two IgG fractions of interest for the understanding of the pathogenesis of rheumatoid arthritis (RA) have been isolated in pure state The fractions referred to are 1) the haemagglutinating fragment of the rheumatoid factor (RF) and 2) a fraction of anti RF serum which in non aggregated state completely neutralizes the RF Some properties of these fractions are listed in Table I

The haemagglutinating fragment causing haemagglutination in the sheep cell test was detected about five years ago (J A M A 194 516 1965 and elsewhere) The fragment was called RRF and was isolated either from RA serum or from the purified rheumatoid factor

As regards the other new member of the large IgG group here referred to an account was given in this journal 185 21 1969 This fraction was called IgR. As pointed out above it has the property of completely neutralizing the RF It goes without saying that the IgR was immediately tried as an antigen and it showed a definite property of provoking a haemagglutinating macroglobulin of type RF in rats These experiences have been described in Proc Symp Immun March 69 Acta path Microbiol scand 77 347 1969 and in this journal 186 135 1969

The presence of the two abovementioned IgG in the joint fluid of RA was tested

As regards the haemagglutinating fragment (RRF) of the rheumatoid factor it was easy to detect its presence in joint fluid from RA by immunoelectrophoresis The next step was to compare the precipitation lines between RRF and anti RRF (produced in our laboratory) as regards joint fluid and serum from the same patient. Through these experiments it was obvious that the precipitation line of RRF versus anti RRF was occasionally somewhat stronger with joint fluid than with serum (Fig 1) This observation gave rise to theoretical considerations and extensive trials are in progress in this field Among other things the variations of RF itself in joint fluid and serum have been studied It was found that the RF versus anti RF precipitation sometimes was stronger in joint fluid than in serum In other trials no difference was observed but rather often the precipitation was somewhat weaker from joint fluid than from serum as may

Table I *Types of 7S gamma globulin tested for presence in serum and joint fluid*

| New terms | Mol. weight | Sedim const. | Electroph mobility | Kappa and lambda light chains | Amount in serum (adults) | Characteristic properties |
|---|---|---|---|---|---|---|
| IgR | 180 000 | 6–7S | Fast γ | Yes | | Complete neutralization of RF (rheumatoid factor) |
| RRF | 200 000 | 6 5–7S | Slow γ | Yes (kappa reaction stronger) | 1–3 mg% (great variations) | Haemagglutinating fragment of RF |

siderably higher yield of positive findings than a random screening program of a population The psychological readiness and motivation to accept life long treatment is also definitely much higher in these relatives of hypertensives than in cases found during a general health survey Plans are presently being made to systematize this policy of case finding on a hereditary basis which we think definitely more worth while than large general screening programs that might bring forth great numbers of psychologically unprepared patients who could hardly be efficiently coped with by our present resources

## REFERENCES


1 Barnett A J Treatment of severe arterial hypertension Results from long term use of methonium compounds with and without the addition of reserpine Aust Ann Med 5 274 1956

2 Bengtsson U & Hood B Abuse of phenacetin containing analgesics and renal damage In Progress in pyelonephritis (ed E H Kass) F A Davis Co Philadelphia 1965

3 Berkson J & Gage R P Calculation of survival rates for cancer Proc Mayo Clin. 25 270 1950

4 Bjork S Sannerstedt R Angervall G & Hood B Treatment and prognosis in malignant hypertension Clinical follow up study of 93 patients on modern medical treatment Acta med scand 166 175 1960

Bjork S Sannerstedt R Falkheden T & Hood B The effect of active drug treatment in severe hypertensive disease An analysis of survival rates in 381 cases on combined treatment with various hypotensive agents Acta med scand 169 673 1961

6 Breslin D J Gifford R W Jr & Fairbairn J F Essential hypertension A twenty year follow up study Circulation 33 87 1966

7 Dustan H P Schneckloth R E Corcoran A C & Page I H The effectiveness of long term treatment of malignant hypertension Circulation 18 644 1958

8 Farmer R G Gifford R W & Hines E A Effect of medical treatment of severe hypertension A follow up study of 161 patients with group 3 and group 4 hypertension Arch intern Med 122 164 1963

9 Harington M Kincaid Smith P & McMichael J Results of treatment in malignant hypertension A 7 year experience in 94 cases Brit. Med J 2 969 1959

10 Harington M Malignant hypertension Practitioner 193 35 1964

11 Hodge J V McQueen, E. G & Smirk H Results of hypotensive therapy in arterial hypertension based on experience with 497 patients treated and 156 controls observed for periods from 1 to 8 years Brit Med J 1 1 1961

12 Hood B Five and a half years experience of combinations of hypotensive drugs. Main present difficulties In Hypotensive drugs a symposium, p 135 (ed M Harington) Pergamon Press, London 1956

13 *Hood B* Bjork S Sannerstedt R, & Angervall G Analysis of mortality and survival in actively treated hypertensive disease Acta med scand 174 393 1963

14 Hood B Aurell M Falkheden T & Bjork, S Analysis of mortality and survival in actively treated hypertensive disease In Antihypertensive therapy (Principles and practice) An international symposium (ed. F Gross) p 370 Springer Verlag Berlin Heidelberg New York 1966

15 Hood B Falkheden T & Carlsson M Trends and present pattern of mortality in chronic uremia Acta med scand 181 561 1967

16 Kincaid Smith P McMichael J & Murphy E. A. The clinical course and pathology of hypertension with papilloedema (malignant hypertension) Quart. J Med 27 117 1958

17 Keith N M Wagener H P & Barker N W Some different types of essential hypertension Their course and prognosis Amer J med Sci 197 332 1939

18 McMichael J Results of methonium treatment in malignant hypertension Five years follow up In Hypotensive drugs (ed M Harington) p 105 Pergamon Press London 1956

19 Mohler E R Jr & Freis E D Five years survival of patients with malignant hypertension treated with antihypertensive agents Amer heart J 60 329 1960

20 North J D K Williams J C P & Howie R N Severe hypertension treated with ganglion blocking drugs in a general hospital Brit Med J 1 14 6 1961

21 Page I H A clinical study of malignant hypertension Ann intern Med 12 978 1939

22 Palmer R. A & Muench H Course and prognosis of essential hypertension Follow up of 453 patients 10 years after original series was closed J Amer med Ass 153 1 1953

23 Peet M M & Isberg E. M The problem of malignant hypertension and its treatment by splanchnic resection Ann intern Med 28 755 1948

24 Perry H N Jr & Schroeder H A The effect of treatment on mortality rates in severe hypertension A comparison of medical and surgical regimens Arch intern Med 102 418 1958

25 Simpson F O & Gilchrist, A R. Prognosis in untreated hypertensive vascular disease Scot. med J 3 1 1958

26 Smirk, F H Principles and details of hypotensive therapy In Hypotensive drugs (ed M Harington) p 109 Pergamon Press London 1956

27 Sokolow M & Perloff D Five years survival of consecutive patients with malignant hypertension treated with antihypertensive agents Amer J cardiol 6 858 1960

28 Veterans administration cooperative study group on antihypertensive agents Effects of treatment on morbidity in hypertension. Results in patients with diastolic blood pressure averaging 115 through 1 9 mm Hg J Amer med ass. 202 116 1967

Acta med scand Vol 187 pp 305-307 1970

# A CASE OF ASYMPTOMATIC JUVENILE DIABETES MELLITUS WITH SEVERE INSULIN DEFICIENCY

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*Abstract* A case of asymptomatic diabetes mellitus in a 13 year-old boy is reported. No significant endogenous insulin response could be induced by four different beta cell stimulatory tests. This insulin pattern is compared with the normal response of the patient s father who was investigated owing to a childhood history of glucosuria The significance of the present case of juvenile diabetes mellitus is discussed in relation to the recently described young subjects with mild and asymptomatic diabetes mellitus.

Juvenile diabetes mellitus is usually characterized by an abrupt onset days to a few weeks pronounced thirst, polyuria nocturia loss of weight, hunger high blood sugar and ketoacidosis (11) This characterization covers the classical concept of diabetes mellitus in young subjects and a few years ago the accepted view was that mild diabetes in subjects under 30 was extremely infrequent (10)

In later years however it has been recognized that an impaired glucose tolerance may exist in children and young adults without presenting symptoms of diabetes mellitus (1 4 7 8) To express the contrast to classical juvenile diabetes such terms as chemical diabetes preclinical diabetes subclinical diabetes latent diabetes or mild juvenile diabetes have been used (1 3 7 8) However more information is obtained by differentiation between asymptomatic diabetics with only minor abnormalities in the glucose tolerance curve called glucose tolerance test diabetics" and patients with permanent 24-hour hyperglycaemia (5) Data on plasma insulin levels in asymptomatic juvenile diabetes have only been reported in a few cases (1 4 7) In these patients the insulin concentrations in serum varied from hyperinsulinism to abnormally low levels Only Hales has reported a case with a transition from mild to classical juvenile diabetes a boy who four years before the onset of ketoacidotic diabetes had a slightly impaired glucose tolerance but normal plasma insulin levels (4) Otherwise the progress and relation to other diabetes types are unknown. The significance of these preclinical diabetes cases in the study of the pathogenesis of diabetes mellitus is not clear For this reason details on the actual case are presented

## CASE REPORT

The patient is a 13 year-old boy Glucosuria was found during routine examination in the school on February 12, 1969 A following oral glucose tolerance test unveiled diabetic glucose tolerance

No diabetes was known in the family The father however a 37 year-old healthy man of normal weight, had a childhood history of glucosuria Since maturity no trace has been seen Because of this history the father underwent the same beta-cell stimulatory tests as did the patient.

The boy had normal physical activities, did not suffer from fatigue thirst or polyuria, nor has there been weight loss or infection prior to admission.

On the day of admission March 4 1969 the boy seemed healthy with normal hydration. His height was 165 cm and weight 53 kg, increasing to 54 5 kg after 4 days without treatment. There were no disturbances of sensation Ophthalmoscopy and electrocardiography were normal The boy was rather muscular with signs of progressing puberty

Blood sugar level was permanently increased during all the 24 hours with the following means and ranges 8 a.m., 19? (138- 65) mg/100 ml, 1 a.m., 370 (2?6-530) mg 100 ml 4 p m. 3?8 (174-600) mg/100 ml, and 8 p m. 302 (273-340) mg/100 ml The glucosuria varied between 3 8 and 7 6 g/100 ml urine

The ?4-hour urine volume varied between ?00-1000 ml during the stay in hospital. During the 4? days hospital there were no acetone bodies in the u ard

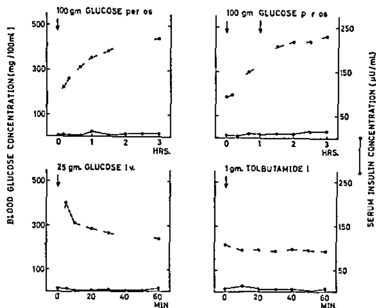



*Fig 1* Blood glucose and serum insulin variations in the patient after loading with glucose and tolbutamide

bicarbonate in blood was 22 0 24 0 and 24 2 mEq/l on three occasions Haemoglobin 157 g/l ESR 2 mm/h serum creatinine 8 mg/l and normal levels of sodium potassium and chloride were noted in the serum

In order to establish the diabetes type with reference to treatment blood glucose and immunoreactive insulin (IRI) were studied during stimulation of beta cells with glucose and tolbutamide The four tests (see Figs 1 and ?) were performed in the morning after 12 hours of Venous blood was drawn from the antecubital Blood glucose was determined using the enzymatic of Christensen (2) and IRI on the principle of Yalow and Berson (12) by the method of Ørskov (13)

As indicated by Fig 1 there was severe insulin deficiency in the boy No significant response to beta-cell stimulation could be measured The serum insulin concentration varied between 1 and 14 microunits/ml, and the fasting values were 6 6 4 and 5 microunits/ml on four occasions (The sensibility of the insulin radioimmunoassay in our laboratory is 2 microunits/ml when the IRI concentration is below 100 microunits/ml) The patient's father had normal carbohydrate tolerance and showed a normal response to insulin stimulation (Fig ?)

With this insulin deficiency in the boy and without insulin response to i v tolbutamide a treatment with diet (?00 g carbohydrate) and Insulin Retard® was started on April 27 1969 Great improvement of both glucosuria and fasting blood glucose concentration was obtained

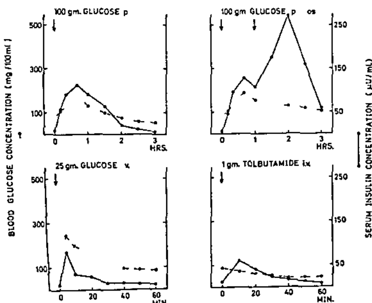



*Fig 2* Blood glucose and serum insulin variations in the father of the patient after loading with glucose and tolbutamide

with 0.9 ml+0.4 ml Insulin Retard®. The insulin treatment was given with the purpose of preventing sudden and serious ketoacidosis.

Outpatient control for five months after discharge showed an unchanged clinical state. The patient was without symptoms. Random blood sugar is 77 mg/100 ml. No episodes of hypoglycaemia have been reported.

## DISCUSSION

The patient described was in a proper clinical sense healthy and in the two-and a half months that he was observed before treatment no symptoms evolved. In spite of this his low and fixed plasma insulin in the presence of extreme degrees of hyperglycaemia resembled classical juvenile diabetes (11) and with glucosuria and fasting hyperglycaemia, his diabetes must be considered manifest (9).

Using determinations of plasma immunoreactive insulin under various loadings and islet-cell stimulations one can get an estimate of the beta-cell function. In the present case an almost complete *beta-cell insufficiency* seems to exist otherwise connected with acute diabetic symptoms which if not treated progress to dangerous ketoacidosis. In itself it is however incomprehensible that the reported degree of glucose-insulin derangement can exist for months or years without producing any symptoms.

Chiumello et al. (1) have recently reported four cases of preclinical diabetes in children aged two to nine years. Two of them, both of normal weight, had a pronounced insulin deficiency although the plasma insulin level was a little higher than in the present case. The two other children, of whom one was 42 % overweight, had a delayed hyperinsulinar pattern as in maturity-onset diabetes.

Except for the two insulin-deficient children described by Chiumello et al. (1) the insulin response in other reported cases of mild juvenile diabetes differs from the present case. Johansen (5) and *Johansen and Lundbæk* (7) have collected a group of young non-obese diabetics with mild glucose intolerance as most often seen in maturity-onset diabetics. Their plasma insulin shows a clear rise after tolbutamide and glucose stimulation. However the insulin response is significantly lower than in maturity-onset diabetics with the same degree of carbohydrate intolerance (6). The authors have pointed out that they did not know whether the patients were in an early phase of classic juvenile diabetes or would later become maturity-onset diabetics.

In the aforementioned case reported by Hales (4) plasma insulin concentrations were almost normal in the investigated asymptomatic stage.

The few reports of plasma insulin in asymptomatic young diabetics have produced a rather dissimilar picture of the beta-cell state. This may be attributed to the fact that the patients were investigated during different stages of the evolution of classical juvenile diabetes mellitus or that the diabetic disease may follow several courses. To elucidate these questions plasma insulin determinations in every case of asymptomatic juvenile diabetes must be of importance.

## REFERENCES


1. Chiumello G., Del Guercio M. J. & Bidone G.: Le diabète chimique ou préclinique à l'âge infantile. Helv. paediat. Acta 23: 11, 1968.
2. Christensen, N. J.: Notes on the glucose oxidase method. Scand. J. clin. Lab. Invest. 19: 379, 1967.
3. Fajans, S. S. & Conn, J. W.: On the nature and treatment of diabetes, p. 641. Excerpta Medica Foundation, Amsterdam 1965.
4. Hales, C. N.: Plasma levels of glucose, nonesterified fatty acid, glycerol and insulin four years before the onset of diabetic ketosis. Lancet 2: 389, 1967.
5. Johansen, K.: Mild diabetes mellitus in children and young adults. Nord. Med. 30: 1080, 1968.
6. — Different plasma insulin response to glucose and tolbutamide in young and old people with the same degree of carbohydrate intolerance. Abstract in Program from Fifth Annual Meeting in Scand. Soc. Diab. Research, p. 21. Copenhagen 1969.
7. Johansen, K. & Lundbæk, K.: Plasma insulin in mild juvenile diabetes. Lancet 1: 1257, 1967.
8. Lister, J.: The clinical spectrum of juvenile diabetes. Lancet 1: 386, 1966.
9. Luft, R., Cerasi, E. & Östman, J.: Prediabetes – latent diabetes – early diabetes. Nord. Med. 80: 1353, 1968.
10. Lundbæk, K.: Endokrinologi, p. 210. Munksgaard, Copenhagen 196_.
11. Parker, M. L., Pildes, R. S., Chao, K. L., Cornblath, M. & Kipnis, D. M.: Juvenile diabetes mellitus, a deficiency of insulin. Diabetes 17: 27, 1968.
12. Yalow, R. S. & Berson, S. A.: Immunoassay of endogenous plasma insulin in man. J. clin. Invest. 39: 1157, 1960.
13. Ørskov, H.: Wick-chromatography for the immunoassay of insulin. Scand. J. clin. Lab. Invest. 20: 297, 1967.

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# PLASMA INSULIN AFTER TOLBUTAMIDE IN DIABETICS AND NON DIABETICS

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*Abstract* Following intravenous injection of 1 0 g tolbutamide the serum insulin and glucose levels have been determined in 27 normal subjects and 11 patients with stable diabetes mellitus, all non-obese All the normal subjects showed an abrupt increase in the concentration of serum insulin, reaching a peak 2 min after the injection Young persons gave a greater insulin response than elderly but the difference was not significant. Patients with stable diabetes gave very little insulin response but in most cases the maximum insulin concentration was also attained in 2 min. This finding, viz. that in diabetics the increase in the insulin concentration following tolbutamide stimulation occurs immediately while after an i.v glucose stimulation it is delayed indicates that the target of tolbutamide in the islet tissue of the beta cells differs from that of glucose

Determination of serum insulin following i v injection of tolbutamide is used in the investigation of patients with spontaneous hypoglycaemia and patients with stable diabetes mellitus However the test is of importance only in assessing non obese patients since the insulin response of obese normal subjects to an i v tolbutamide load is usually indistinguishable from that of patients with insulinoma (15)

The object of this study was to present a normal material for evaluating the insulin secretion following i.v injection of 1 g sodium tolbutamide Furthermore the tolbutamide induced insulin response in patients with stable diabetes mellitus was determined This was done in order to ascertain whether the sluggishness of insulin secretion which is characteristic of non-obese diabetics tested with glucose is also manifest upon stimulation of the beta cell with tolbutamide (14)

## MATERIAL AND METHODS

The normal group comprised 27 persons of the hospital staff or patients with mild diseases which did not affect their general condition (neuroses, arthralgia, gastritis) All the subjects were normal weight (mean weight ±10 ) assessed on the basis of Natvig s height weight tables (12) All were ambulatory had a Hb concentration exceeding 12 g 100 ml a B P of less than 150 100 mm, serum creatinine < 1 2 mg 100 ml, fasting plasma glucose level < 110 mg/100 ml and an ESR < 15 mm/h except for one patient whose ESR was 40 mm/h None had glycosuria or haematuria, and only one patient had proteinuria—of unknown cause None had diabetes mellitus assessed by i v glucose tolerance using 25 g *d* glucose and/or i.v tolbutamide tolerance test with 1 g sodium tolbutamide Seventeen of the normal subjects (8 women and 9 men) were less than 45 years of age (average 32) ten (4 women and 6 men) were over 45 (average 54 years)

The diabetic group comprised 11 patients with stable diabetes six women and five men, average age 65 years. Further clinical and haematological data are listed in Table I All the patients were suffering from clinically manifest diabetes mellitus, i e hyperglycaemia and glycosuria and all were non-obese according to Natvig s tables (12) All the patients were ambulatory and their diabetes was well-controlled.

The tests were carried out at 8–10 a.m after about 14 hours fasting. With the patient recumbent a Mediplast needle was inserted into the cubital vein and through this needle all the blood samples were drawn by a syringe without using a tourniquet After the removal of 1 or 2 fasting samples, 1 g sodium tolbutamide was injected in the course of 1 min The glucose concentration was determined in capillary blood by the method of Hagedorn–Norman Jensen or in capillary plasma by the glucose oxidase method 2, 5 10 20 30 and 60 min after the injection of tolbutamide Serum insulin was determined immunologically by a modification of the method of Hales and Randle (4) at the same junctures ($^{125}$I hog insulin supplied by the Steno Memorial Hospital, Gentofte Copenhagen) All analyses were done in duplicate The analytical reproducibility stated as the coefficient of variation $100 \times s/m$ was 2.9% for the glucose determination and 9 4% for the insulin determination. There was no difference between the plasma glucose determination performed by the glucose oxidase method and the blood sugar determination on whole blood by the Hagedorn–Norman Jensen method.

Table I *Data concerning the 11 non obese patients with maturity onset diabetes*

| Case no | Age (y) | Sex | Complicating diseases | Hb (g/100 ml) | ESR (mm/h) | Urine Protein | Urine Blood | BP (mm Hg) | Serum creatinine (mg/100 ml) | Duration of diabetes (y) | Treatment (daily) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 64 | ♀ | Nephropathy Breast tumour | 13 6 | 10 | + | – | 160/70 | 0 80 | 41 | Chlorpropamide 250 mg × 2, Phenformin 50 mg × 2 |
| 2 | 75 | ♀ | R sided hydronephrosis Hiatal hernia | 13 5 | 25 | — | — | 150/80 | 0 90 | 1/12 | Tolbutamide 1 0 g |
| 3 | 78 | ♂ | Osteoarthritis of r knee | 16 4 | 2 | — | — | 160/90 | 0 91 | 4/12 | Tolbutamide 0 5 g × 2 |
| 4 | 45 | ♀ | Occlusion of r fem art | 15 5 | 20 | — | — | 170/100 | 0 84 | 15 | Tolbutamide 0 5 g |
| 5 | 38 | ♂ | Degen of i v disc (lumb ) | 13 5 | 40 | — | — | 150/90 | 0 80 | 1/12 | Insulin for 2 days |
| 6 | 54 | ♂ | L sided nephrolithiasis | 14 0 | 30 | + | – | 110/60 | 1 13 | 1 | Tolbutamide 0 5 g × 2 |
| 7 | 70 | ♀ | Art scl heart disease | 13 5 | 10 | — | — | 145/90 | 0 78 | 10 | Tolbutamide 1 0 g |
| 8 | 74 | ♂ | Cerebral haemorrhage Diabetic retinopathy | 14 0 | 4 | — | — | 210/100 | 1 02 | 1 | Tolbutamide 1 0 g |
| 9 | 72 | ♀ | Art scl heart disease Art scl of legs | 12 9 | 27 | — | — | 190/80 | 1 06 | 17 | Tolbutamide 0 5 g |
| 10 | 60 | ♂ | Art scl heart disease | 14 6 | 5 | — | — | 120/70 | 0 88 | 7 | Insulin for 5 days |
| 11 | 79 | ♀ | Art scl heart disease | 13 8 | 13 | — | — | 110/90 | 1 00 | 4 | Phenformin 50 mg × 2 |

## RESULTS

results are given in Figs 1 2 and 3 The lucose determinations confirm the well known fact that the fall in plasma glucose concentration following an i v injection of tolbutamide is more delayed in diabetics than in normal subjects Determinations of serum insulin showed 1) that the release of insulin from the beta cell occurs immediately after the injection of tolbutamide 2) that in patients with stable diabetes this release of insulin from the beta cell also takes place immediately after the injection but that in these patients the increase in the serum level of insulin is considerably lowered especially when the diabetes is of many years duration and 3) that the increase in the serum level of insulin is not significantly less marked in elderly than in younger normal subjects (cf Fig 3)

Only in one case did the tolbutamide test have to be interrupted because of neuroglucopenia

## DISCUSSION

It has been substantiated by experiments in vivo as well as in vitro that tolbutamide stimulates the beta cell to increased release of insulin (1 11) The target of tolbutamide in the beta cell is a matter of discussion However it is apparent from in vitro experiments that tolbutamide stimulates the secretion of insulin in a way different from that of glucose the secretion after tolbutamide being uninhibited even under circumstances in which glucose induced insulin secretion will be blocked by mannoheptulose (1) The same is indicated by experiments using diazoxide which inhibits glucose induced but not tolbutamide induced secretion of insulin (5)

The abrupt increase in insulin concentration immediately after injection of tolbu amide corresponds to the findings of o hers (9 11) Assessment of the insulin secretion by serum analyses 30–60 min after injection of tolbutamid as used previously (16) must therefore be considered insufficient

It is remarkable that in many cases the insulin level has become almost normal at the time when the hypoglycaemia is most pronounced This is in keeping with experience from patients with insulinoma whose insulin level is not infrequently

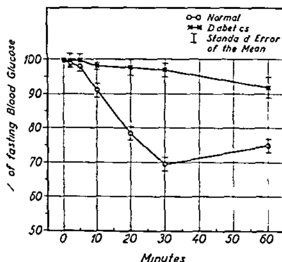



*Fig 1* Plasma glucose in % of fasting plasma glucose level following i.v. injection of 1 g sodium tolbutamide into 27 normal weight non-diabetics (O) and 11 normal weight patients with stable diabetes mellitus (×). Mean value ±S.E.M. stated

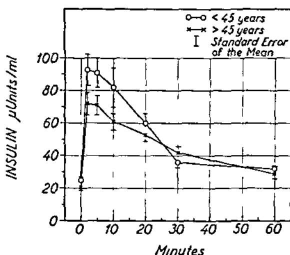



*Fig 3* Serum level of insulin following i.v. injection of 1 g sodium tolbutamide into normal weight non-diabetics, 17 persons under 45 years of age (average 32 years) (O). 10 persons over 45 years (average 54) (×). The mean value ±S.E.M. is stated. The differences at the various times are not significant ($p > 0.05$)

normal even at a time when they exhibit hypoglycaemic attacks.

From the present experiments it is evident that among elderly non obese persons several give a poorer insulin response than younger subjects to tolbutamide. However the scatter of the results is considerable and the demonstrated difference in insulin secretion between youngish and older normal persons is not significant. Indeed Jørgensen (8) found no age difference in the secretion of insulin following oral glucose tolerance in non obese non diabetics.

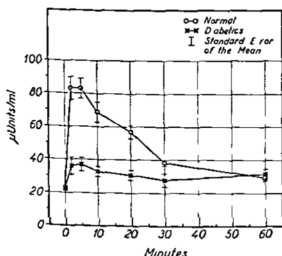



*Fig 2* Serum level of insulin following i.v. injection of 1 g sodium tolbutamide into 27 normal weight non-diabetics (O) and 11 normal weight patients with stable diabetes mellitus (×). Mean value ±S.E.M. stated. The differences at 2, 5, 10, 20 and 30 min are significant ($p < 0.001$, for 30 min $< 0.02$)

The cause of the poor insulin response in the diabetic group is unknown. It is not likely to be due exclusively to the decreased mass of beta cells in patients with stable diabetes (6) since the insulin response of diabetics to secretin is normal (3). It is more likely to be a confirmation that the enzymatic beta cell mechanisms involved in the secretion of insulin are deranged in diabetics. The rapid increase in insulin secretion following tolbutamide in diabetics too must be taken to mean that the passage of the insulin molecule from the beta cell to the capillaries is intact. It is remarkable that the sluggishness in insulin secretion in diabetics following stimulation with glucose is not manifest after tolbutamide. Similar findings have been reported by Kipnis (9) and Melani (11) and must be considered another proof that tolbutamide affects the secretion of insulin through other paths than does glucose.

It is unlikely that the very slight increase in insulin concentration following tolbutamide in diabetics is able to explain the effect of tolbutamide upon the glucose turnover in these patients

There are several indications that apart from its insulogenic effect tolbutamide also exerts an effect upon the liver and muscle cells, sensitizing them to insulin (2, 10).

I.v. tolbutamide test followed by glucose and insulin determinations is a useful test in investigating hypoglycaemic conditions in non-obese persons, as more than 80% of patients with insulinoma show an abnormally high and/or long lasting insulin response to tolbutamide while abnormally high insulin concentrations are rarely seen during tolbutamide tolerance tests in other diseases which may give rise to hypoglycaemia (except for cirrhosis). However, it should be stressed that in a few healthy non-obese young persons abnormally high insulin concentrations (> 200 μ units/ml) may be observed following i.v. injection of tolbutamide. A high insulin response may also be found in obese patients, patients with cirrhosis, hypercorticism, lipo-atrophic diabetes and acromegaly. Accordingly this test is indicated in patients with reduced glucose tolerance whose diabetic syndrome is believed to be due to cirrhosis, pituitary disorder or hypercorticism. In all other types of diabetes mellitus the secretion of insulin is abnormally low following tolbutamide (7).

ACKNOWLEDGEMENT

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## REFERENCES

1. Coore, H. G. & Randle, P. J.: Regulation of insulin secretion studied with pieces of rabbit pancreas incubated in vitro. Biochem. J. 93: 66, 1964.
2. de Belle, R., Belmonte, M. M. & Colle, E.: Effect of intravenous tolbutamide in juvenile diabetes mellitus. Diabetes 16: 215, 1967.
3. Deckert, T.: Insulin secretion following administration of secretin in patients with diabetes mellitus. Acta endocr. (Kbh.) 59: 150, 1968.
4. Deckert, T. & Hagerup, L.: Serum insulin in normal and obese persons. Acta med. scand. 182: 225, 1967.
5. Fajans, S. S., Floyd, J. C., Thiffault, C. A., Knopf, R. F. & Conn, J. A.: Mechanisms involved in the hyperglycemic action of diazoxide. In: Diabetes, Proceedings of the Sixth Congress of the International Diabetes Federation, pp. 887–893. Excerpta Medica Foundation, Amsterdam 1969.
6. Gepts, W.: Die Pathogenese des Diabetes Mellitus, p. 42. Springer, Berlin, Heidelberg, New York 1967.
7. Johansen, K. & Lundbæk, K.: The plasma insulin response to glucose and tolbutamide in mild juvenile diabetes mellitus. In: Tolbutamide, after ten years, pp. 255–260. Excerpta Medica Foundation, Amsterdam 1967.
8. Jørgensen, K. R.: Evaluation of the double antibody radioimmunoassay of insulin and the determination of insulin in plasma and urine in normal subjects. Acta endocr. (Kbh.) 60: 327, 1969.
9. Kipnis, D. M.: Insulin antagonism and diabetes mellitus. In: Diabetes, Proceedings of the Sixth Congress of the International Diabetes Federation, pp. 257–272. Excerpta Medica Foundation, Amsterdam 1969.
10. Madsen, J.: Extrapancreatic and intrapancreatic action of antidiabetic sulphonylureas. A review. Acta med. scand. Suppl. 476, pp. 109–122, 1967.
11. Melani, F.: Seruminsulin concentration following intravenous administration of tolbutamide. Acta Diabetologica Latina Suppl. 1: 106, 1967.
12. Natvig, H.: Nye høyde-vekttabeller for norske kvinner og menn. Landsforeningen for kosthold og helse, Oslo 19.6.
13. Perley, M. J. & Kipnis, D. M.: Plasma insulin responses to glucose and tolbutamide of normal weight and obese diabetic and non-diabetic subjects. Diabetes 15: 867, 1966.
14. — Plasma insulin response to oral and intravenous glucose. Studies in normal and diabetic subjects. J. clin. Invest. 46: 1954, 1967.
15. Samols, E. & Marks, V.: Evaluation of the intravenous tolbutamide test in the recognition and differential diagnosis of spontaneous hypoglycemia. In: Tolbutamide, after ten years, p. 147. Excerpta Medica Foundation, Amsterdam 1967.
16. Yalow, R. S., Black, H., Villazon, M. & Berson, S. A.: Comparison of plasma insulin levels following administration of tolbutamide and glucose. Diabetes 9: 356, 1960.

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# PHEOCHROMOCYTOMA

*A Review of Clinical Findings in Ten Cases*

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*Abstract* In a series of ten patients a total of 14 pheochromocytomas have been removed All the tumors were located in the adrenal glands except for one situated in the organ of Zuckerkandl. In one man two tumors were removed from the right adrenal gland with an interval of five years Bilateral pheochromocytoma was found in three of five young women In two of them the second tumor appeared as late as seven and ten years respectively after removal of the first. A woman got her second pheochromocytoma during pregnancy She was treated with alpha methyldopa and went through an uncomplicated delivery A fortnight later the tumor was removed Two men suffering from diabetes mellitus were ultimately diagnosed as having pheochromocytoma. Removal of the tumor led to cure The same applied to two young women suffering from acute and chronic nephritis" respectively In two instances the urinary output of catecholamines was normal, while the excretion of metabolites was significantly elevated In another instance the excretion of vanillin mandelic acid was normal whereas the remaining biochemical outputs were increased

Pheochromocytoma is of continuous interest as a rare (6) but remediable cause of hypertension However pheochromocytoma may also present under other clinical pictures which should receive as much attention as the hypertensive disease

This report is a reminder that pheochromocytoma can be the curable cause of diabetes mellitus as well as of *nephritis*

## MATERIAL

The present series comprises the cases of pheochromocytoma admitted to our department during the 10 year period from 1959 to 1968

The patients went through the routine clinical examinations including renal arteriography and urine determination of the catecholamine output. The latter were considered so safe and convenient that provocation tests were omitted (5 8) A phentolamine test was, however consistently carried out.

## RESULTS

The series revealed some differences between the sexes (Table I) The women were on the average much younger and four of the five were less than twenty years old Their weight was normal while all the men were overweight

A total of 14 pheochromocytomas were removed in the ten patients (Table II) Only one of the tumors was extra adrenal and located in the organ of Zuckerkandl In a man aged 31 two pheochromocytomas were removed from the right adrenal gland with an interval of five years The last tumor was irregularly encapsulated but without signs of malignancy Bilateral pheochromocytoma was present in no less than three of the five women In one of these the first tumor was *removed at the age of 6 the second from the* contralateral adrenal gland at the age of 16 Another female patient underwent operation for a pheochromocytoma of the left adrenal at the age of 13 During pregnancy seven years later another pheochromocytoma was diagnosed The hypertension was controlled by means of a thiazide and alpha methyldopa and the delivery was uneventful A couple of weeks later the second tumor *was removed from the right adrenal* In a third woman aged 19 years multiple small tumors were simultaneously removed from both adrenal glands

Hypertensive encephalopathy was responsible for the most prominent subjective complaints (Table III) Two patients suffered from syncopal attacks a girl of 6 years had epileptic seizures while a man had experienced episodes of transient hemiparesis Symptoms from the heart and vessels were less conspicuous Two men complained of chest pain unrelated to effort Tachycardia oc

Table I *Mean values for age weight and height in ten patients with pheochromocytoma*

| Sex | No | Age | Weight | Height |
|---|---|---|---|---|
| Men | 5 | 45 | 79 | 174 |
| Women | 5 | 27 | 56 | 160 |
| Total | 10 | 35 | 68 | 167 |

Table II *Localization of 14 pheochromocytomas in ten patients*

| | | Adrenal | | |
|---|---|---|---|---|
| Sex | No | Left | Right | Extra adrenal |
| Men | 5 | 1 | 4 | 1 |
| Women | 5 | 4 | 4 | 0 |
| Total | 10 | 5 | 8 | 1 |

curred in some patients but only for short periods of time A notable observation was made by a medical male student, whose pulse rate during running rose to about 140 and then suddenly used to drop to half of that frequency Simultaneously he experienced headache goose flesh and profuse [illegible] Also other patients stated that exercise [illegible] red the onset of symptoms Real constipation was troublesome in only one patient but abdominal discomfort as noted by many others probably was due to constipation In contrast one man presented with a severe colitis and bloody stools

Sustained hypertension was present in all but one of the patients (Table IV) Periodical increases of the blood pressure were observed in all Next to hypertension the metabolic symptoms were most conspicuous Glucosuria although intermittent proved to be a significant finding In one patient an abnormal glucose tolerance was found in the absence of glucosuria and with normal fasting blood sugar Of essential interest were two men suffering from frank diabetes mellitus treated with chlorpropamide and regular insulin respectively The presence of hypertension and the absence of keto-acidosis ultimately led to the correct diagnosis. Removal of the pheochromocytoma cured their diabetes as well as their hypertension

Proteinuria was a frequent finding, but only two patients had cylindruria and hematuria One of these was a girl of 19 who was admitted to our department with chronic nephritis and concomitant hypertension Her complaints were constipation headache and sweating Continuous proteinuria had been present since the age of 10 The examination was unrewarding except for the presence of protein and some granular casts in the urine. She had normal eyegrounds normal ECG and normal urography Tests of the renal function were normal as was the glucose tolerance and the urinary output of catecholamines She was discharged on thiazide medication About four months later she was readmitted for further investigation including renal angiography and renal biopsy Her blood pressure had then increased to 175/130 mm Hg and treatment with bethanidine sulfate was initiated This occasioned however an alarming response (14) In the course of a few hours the blood pressure rose to 265/165 and the pulse rate to 160 and she felt seriously ill with headache sweating and vomiting. The treatment was immediately discontinued and a subsequent phentolamine test produced a significant fall of the blood pressure Renal angiography revealed bilateral adrenal enlargement The excretion of catecholamines in the urine was

Table III *Subjective complaints in ten patients with pheochromocytoma*

| Complaints | No |
|---|---|
| Headache | 8 |
| Dizziness | 7 |
| Palpitations | 7 |
| Blurred vision | 6 |
| Abdominal pain | 5 |
| Lassitude | 5 |
| Sweating | 4 |
| Nausea/vomiting | 3 |
| Weight loss | 3 |

Table IV *Clinical findings in ten patients with pheochromocytoma*

| Clinical findings | No |
|---|---|
| Sustained hypertension | 9 |
| Decreased glucose tolerance | 7 |
| Incr fasting blood sugar | 6 |
| Glucosuria | 6 |
| Proteinuria | 6 |
| Cylindruria | 3 |
| Hematuria | 2 |
| Overt diabetes mellitus | • |

still normal This time however the output of metabolites was also examined and found to be much above normal After removal of several tumours in both adrenals the renal disease disappeared, while the blood pressure did not instantaneously reach normal levels

In another girl of 16 who was admitted for acute nephritis the prominent findings were a blood pressure of 180/130 mm Hg eyegrounds with probable papilledema and the presence of protein red blood cells and granular casts in the urine The serum creatinine and non protein nitrogen values were found to be normal Due to intermittent glucosuria and elevated fasting blood sugar it was decided to examine the urinary excretion of catecholamine metabolites A week prior to admission the output of catecholamines in the urine had been found within normal limits Again the excretion of the metabolites was markedly elevated Subsequ nt removal of a pheochromocytoma of the left adrenal gland led to cure of the acute nephritis as well as of the hypertension

Serial estimations of plasma free fatty acids in five patients revealed only normal values The same applied to the measurement of the basal metabolic rate in seven patients A phentolamine test was carried out on ten occasions in seven patients with a mean blood pressure fall of 33/56 mm Hg In one woman the test produced a virtual circulatory shock which was successfully treated with metaraminol

A man aged 63 died of cerebral hemorrhage before any examination could be made The necropsy demonstrated the presence of an adrenal pheochromocytoma

The urinary output of adrenalin and noradrenalin was measured in all the patients (Table V) while the excretion of metabolites was determined in seven The output of adrenalin was significant in all cas s indicating that the tumor was situated in the adrenal gland As mentioned the excretion of catecholamines was normal in two patients whose urinary content of metabolites was markedly elevated In another patient the excretion of catecholamines was abnormally high whereas the excretion of vanillin mandelic acid was normal Unfortunately other metabolites were not studied in this case

Regarding the reliability of the biochemical tests this small series does not permit valid conclusions However it seems reasonable to determine the urinary output of catecholamines and metadrenalin and metanoradrenalin whenever the presence of pheochromocytoma is suspected

Table V *Mean values and ranges for urinary excretion of catecholamines and their metabolites*

Normal values in brackets Catecholamine output in μg/24 h metabolites in μg/mg creatinine

| Adrenalin | Noradrenalin | Vanillin mandelic acid | Metadrenalin | Normetadrenalin |
|---|---|---|---|---|
| 127 | 1144 | 18 8 | 1 02 | 3 7 |
| 4–430 | 55–5080 | 2.5–40 0 | 0 02–2 00 | 0 3–9 0 |
| (< 20) | (< 50) | (< 0 1) | (< 0 1) | (< 0 1) |

## COMMENTS

Pheochromocytoma as the cause of hypertension has received great attention although found in less than 1 % of all cases (6) That this tumor can produce other clinical syndromes has been poorly recognized

Diabetes mellitus caused by pheochromocytoma is probably a rarity but this possibility should always be ruled out. The leading additional symptoms in this resp ct are hypertension and the near absence of keto acidosis The hyperglycemia produced by pheochromocytoma has previously been attributed to an augmented breakdown of liver glycogen Recent research has proved this question to be far more complex with suppression of the insulin release as well as of its peripheral effect (12) Possibly new biochemical tests will emerge from these discoveries

Pheochromocytoma masquerading as acute and chronic nephritis has also received little attention although the occurrence of this tumor in the urinary bladder has been reported (1 10) According to the present report pheochromocytoma can produce a perfect image of nephritis Again the leading symptoms are a conspicuous hypertension and glucosuria

Largely the symptoms produced by pheochromocytoma are rather uniform (10) The high incidence in this series of bilateral pheochromocytoma among young patients is in accordance with other communications (13) but the late appearance of the second tumor must be considered

Removal of a pheochromocytoma in youngsters is an obvious indication for regular control over several years Familial occurrence associated malignancy or malignant tumors were not encountered (5 9 11 15) In view of the fact that normotension was found in only one of the ten patients it should be stressed that the term paroxysmal must be clearly defined (7) Patients with sustained hypertension also present with episodes of sharp increases of the tension often released by effort. *Such cases must not be classified as paroxysmal hypertension*

Pheochromocytoma during pregnancy has been charged with a maternal death rate of 50% (19) and has been considered an indication for immediate surgery (16) The course of the young woman in this report is therefore notable as she went through an uncomplicated delivery by means of routine hypotensive therapy

No increases of plasma free fatty acids or the metabolic rates were observed in this series (3) neither was the hypotensive variety of pheochromocytoma encountered (4) Venous catheterization in localizing the tumor was never considered (2) because the surgery for pheochromocytoma was undertaken with a wide exposure of the ...athetic chain

In the diagnosis of pheochromocytoma the ... nation of the catecholamines and their metabolites remains essential (17 18) Provocative tests are nearly outdated while the phentolamine test is still used as a screening procedure According to this report the best tool for confirming the presence of a pheochromocytoma is the determination of the catecholamines proper plus metadrenalin and metanoradrenalin

## REFERENCES


1 Blair D W & Branwood A.W Phaeochromocytoma of the urinary bladder Brit. J Urol. 35 ,93 1963
2. Crout, J R & Sjoerdsma, A. Catecholamines in the localization of pheochromocytoma. Circulation 22 516, 1960
3 Engelman, K., Mueller P S & Sjoerdsma, A. Elevated plasma free fatty acid concentrations in patients with pheochromocytoma. New Engl J Med. 270 865 1964
4 Hamrin, B., Sustained hypotension and shock due to an adrenaline secreting pheochromocytoma. Lancet 2 123 1962.
5 Harrison, T S., Bartlett, J D., Jr & Seaton J F., Current evaluation and management of pheochromocytoma. Ann. Surg. 168 701 1968
6 Hillestad, L. Etiology in systemic arterial hypertension. Acta med scand 184 225 1968
7 Kirkendall, W M Dale Liechty R. & Culp D A. Diagnosis and treatment of patients with pheochromocytoma. Arch. intern. Med. 115 529 1965
8 Lawrence A M Glucagon provocative test for pheochromocytoma. Ann. intern. Med. 66 1091 1967
9 Nourok, D S Familial pheochromocytoma and thyreoid carcinoma. Ann. intern. Med. 60 10..8 1964
10 Page I B & Copland, R. B Pheochromocytoma. Disease a Month January 1968
11 Sapira J D., Altman, M Vandyk, K. & Shapiro A. P Bilateral adrenal pheochromocytoma and medullary thyreoid carcinoma. New Engl J Med. 273 140 1965
12 Spergel G Bleicher S J & Ertel, N H Carbohydrate and fat metabolism in patients with phaeochromocytoma. New Engl. J Med 278 803 1968
13 Stackpole R. H Melicow M M & Uson, A. C.. Pheochromocytoma in children. J Pediat. 63 315 1963
14 Stafford A Potentiation of some catecholamines by phenoxybenzamine guanethidine and cocaine Brit. J Pharmacol 21 361 1963
15 Tischerman, S E. & Danowski T S Familial pheochromocytoma J Amer med Ass. 182 152, 196..
16 Walker R. M Pheochromocytoma in relation to pregnancy Brit. J Surg. 51 590 1964
17 Williams, R. H Textbook of Endocrinology Saunders Philadelphia 1968
18 Wolf R. L. Mendlowitz, M., Roboz, J & Gitlow S E. Simultaneous urinary assays for the combined metanephrines and 3 methoxy-4 hydroxyphenylglycol in patients with pheochromocytoma and primary hypertension New Engl J Med 273 1459 1965
19 Wulsin, J H Pheochromocytoma in the mesosigmoid following term delivery J Amer med Ass. 190 42, 1964

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# EXERCISE RELEASED VENTRICULAR FIBRILLATION IN HYPERTROPHIC SUBAORTIC STENOSIS TREATED WITH PROPRANOLOL

*A Case Report*

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*Abstract* A 2 year-old woman was controlled for two-and a half years after surgical treatment for hypertrophic subaortic stenosis Exercise tests at identical loads (200 and 400 kpm/min) were performed before and after treatment with propranolol which had been instituted because of angina pectoris After propranolol there was subjective improvement of the chest pain, and the heart rate at rest and during exercise was lower After 4 min at 400 kpm/min (heart rate 154/min) an irregular ventricular tachycardia appeared which changed into several short periods of ventricular fibrillation The patient became unconscious but continued spontaneous breathing during external heart massage given until sinus rhythm spontaneously returned after 3 min The further clinical course was uneventful The possible causal role of propranolol in this arrhythmia is discussed

Beta receptor blocking agents have been recommended for relief of angina pectoris and for the prevention of excessive sympathetic stimulation in hypertrophic subaortic stenosis (2 3 10 12) although this therapy has been of greater subjective than objective benefit Heart insufficiency and obstructive lung disease are generally considered as contraindications Wolfsson (15) also warned against the use of beta receptor agents in patients with defects in the atrioventricular conduction Close observation of patients with muscular subaortic stenosis during beta receptor blocking therapy has been recommended (11) mainly in respect of the risk of decreased cardiac output The fatal complications seen among 2000 cases treated with beta receptor blocking agents have been summarized by Stephen (14) during peroral treatment there were three cases of cardiac failure in patients with hypertrophic subaortic stenosis

This report describes a hazardous cardiac complication during an exercise test in a 22 year old woman operated for hypertrophic subaortic stenosis Postoperatively she had signs of residual stenosis and angina pectoris which was treated with propranolol

## CASE REPORT

The patient, born in Finland in 1946 is the third among four sisters The family history does not include any known cardiovascular disease She had not had diphtheria rheumatic fever or scarlet fever

The patient took part in athletics at school and in skiing competitions until 1962, when she became more tired and dyspnoeic on effort Once during a skiing tour she had a short period of unconsciousness In April 1962 she sought medical advice for the first time because of back pains tiredness and dyspnoea on effort Congenital heart disease was suspected and the patient was admitted to her local hospital where a "high septal defect" was suspected She continued with some skiing until 1963 but did not take part in competitions. Since the autumn of 1962 she had sharp pains on exercise localized to the anterior chest wall tiredness and dyspnoea. During the last year she lost 5 kg in weight

On admission to our hospital in February 1965 the 18 year-old girl was found to be in a good general condition The body constitution and a high palatinal vault aroused suspicion of Marfan s syndrome but there was no lens ectopy Heart slight precordial bulging, no parasternal pulsations The apex beat was double but otherwise normal The heart sounds were normal over the apex and weak over the base The second heart sound was not split. There was a 4th sound over the apex A high tched rather short, grade II mid-systolic diamond shaped murmur was heard over the whole precordium with maximum over the apex The ECG was followed f om 196? (Fig 1) and the physical working capacity

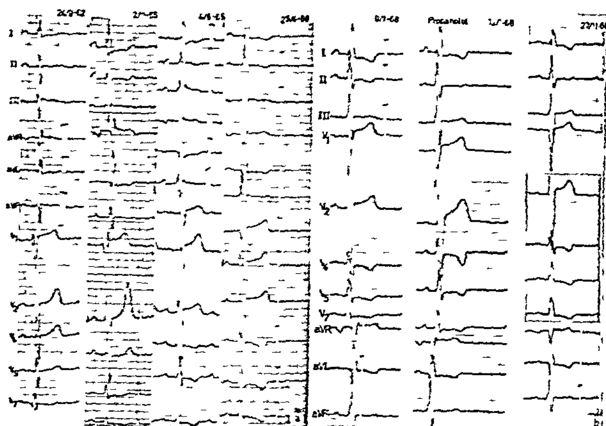



*Fig. 1.* ECG at supine rest. *(a)* Preoperatively no medication, progress of signs of left ventricular and atrial hypertrophy from 1962 to February 1965 and postoperatively digitalis medication, increased ST T changes. *(b)* In January 1968 no medication, after three days of propranolol treatment, shorter PQ duration despite bradycardia and increased ST T changes especially in lead $V_4$, finally ten months later no medication.

from 1965 (Table I). The dynamic spirometry with a light weight spirometer showed slightly decreased values for vital capacity and ventilation capacity (76 % of predicted). The chest radiogram (Fig. 2) showed a normal heart volume, about 600 ml, equalling 410 ml sq.m. body surface area.

At right heart catheterization the pressures were normal, with a pulmonary artery wedge pressure (PAW) of 10 mm Hg. The pressure in the aortic arch was 100 62 (mean 80) mm Hg. At transseptal catheterization a left atrial (LA) pressure of 12 mm Hg was found. Before the left ventricle (LV) could be entered, cardiac tamponade developed with low arterial and high venous pressures. The situation was cleared up by pericardiocentesis. At a later retrograde left ventricular angiography the initial LV pressure was 1.8 12 mm Hg, and after two cineangiographies increased to 190 37 mm Hg. The pressure in the aorta was 160 110 mm Hg, the systolic pressure gradient thus being 30 mm Hg. As there was a strong tendency to arrhythmia the positioning of the catheter tip in the left ventricle was difficult. The LV pressure may thus have been measured above the hypertrophic stenosis in the ventricle (Fig. 3 *a* and *b*).

At operation in May 1965 (V. O. Björk) an excision of the hypertrophic muscle was made in extracorporeal circulation. After aortic incision the myocardium was excised 4 cm below the valve so that a canal 1 cm in depth and 1 cm in breadth was formed. There was no pressure gradient over the aortic valve during operation, either before or after the excision.

At one year follow-up the patient, still on digitalis, was subjectively better. The physical findings were unchanged. In a work test the patient was able to perform 4 min, as compared to 6 min preoperatively, on a work load of 400 kpm min with the same heart rate (Table I). At 400 kpm min the QRS duration became prolonged to 12 csec.

Two-and-a-half years after operation the patient complained of increased chest pains and had not been able to resume work. The ECG at rest is seen in Fig. 1, and the result of a work test in Fig. 4 *a* and in Table I. As her symptoms were interpreted to be angina pectoris, although not typical, propranolol was given, starting with 10 mg × 4 and increasing daily up to 40 mg × 4. The exercise test was then repeated (Fig. 4 *b*). During this exercise test the patient had no complaints on a work load of 200 kpm min, nor during the first 4 min on 400 kpm min. The heart rate was significantly lower than three days earlier without propranolol. As in earlier tests, the arterial blood pressure was found to be somewhat lower on the

Table I *Results of the preoperative and several postoperative exercise tests performed sitting on a bicycle ergometer at identical work loads of 200 and 400 kpm/min (except 10 months after the actual arrhythmia when the loads were 150 and 300 kpm/min) Operation performed on May 20 1965*

| Date of test and therapy | | At rest supine | Standing 8 min | 200 kpm/min | | | 400 kpm/min | | | After work 10 min |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 2 min | 4 min | 6 min | 2 min | 4 min | 6 min | |
| 3 1965 No therapy | HR | 78 | 96 | 146 | 142 | 150 | 178 | 185 | 190 | 100 |
| | RR | 18 | | | 20 | | | 8 | | |
| | BP | 110/50 | 110/70 | | 130 80 | | | 125/70 | | 1 0 /0 |
| | PEF | 390 | | | | | | | | |
| 27 4 1966 Digoxin 0 5 mg daily | HR | 60 | 78 | 138 | 156 | 166 | 182 | 185 | | 86 |
| | RR | 16 | | | 6 | | | 40 | | |
| | BP | 130/ 0 | 125/ 0 | | 125/ 0 | | | 125/ 0 | | 125/70 |
| | PEF | 435 | | | | | | | | |
| 9 1 1968 No therapy | HR | 62 | 75 | 140 | 140 | 148 | 177 | 185 | | 78 |
| | RR | 18 | | | 22 | | | 30 | | |
| | BP | 110 60 | 115 65 | | 120/75 | | | 110/ 0 | | 125 65 |
| | PEF | 480 | | | | | | | | |
| 12 1 1968 Propranolol 160 mg daily since 9 1 1968 | HR | 46 | 53 | 84 | 89 | 91 | 135 | 154 | | 85 |
| | RR | 18 | | | 22 | | | 6 | | |
| | BP | 100/70 | 100 65 | | 115 85 | | | 110 85 | | 110 80 |
| | PEF | 405 | | | | | | | | |
| 27 11 1968 No therapy | HR | 65 | 86 | 111 | 111 | 117 | 150 | 162 | | 68 |
| | RR | 18 | | | 2 | | 28 | 32 | | |
| | BP | 110 65 | 115/70 | | 140/70 | | | 140/70 | | 1 5 65 |
| | PEF | 485 | | | | | | | | |

HR = heart rate RR = respiration rate BP = peripheral blood pressure in mm Hg PEF = peak expiratory flow in l min

second than on the first work load. Suddenly an irregular tachycardia (168 beats/min) with aberrant ventricular ECG complexes, interpreted as ventricular tachycardia, appeared. Initially the patient felt no distress but a few seconds later she became unconscious. The ECG changes had progressed through more obvious ventricular tachycardia to short transient periods of coarse wave ventricular fibrillation. External heart massage was given,

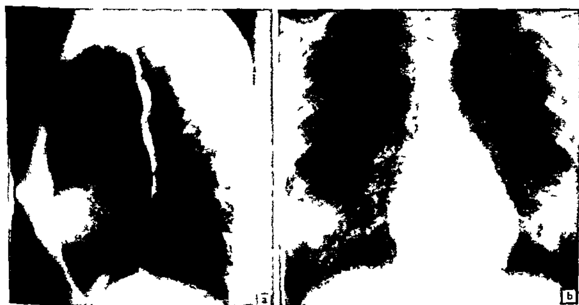



*Fig 2* Chest X ray (*a*) Frontal projection. (*b*) Lateral projection, no signs of selective hypertrophy




Fig 3 Left ventricular retrograde angiography (a) Anterior view (b) Lateral view with muscular subvalvular stenosis 3 cm below the aortic valve and wide coronary arteries

the patient breathing spontaneously About 3 min after the start of the arrhythmia sinus rhythm returned spontaneously For a few days the blood pressure was low 100-95/70-65 mm Hg, and the patient had local pains at the costo chondral junction of a rib There were no further ECG changes and no other signs of myocardial damage

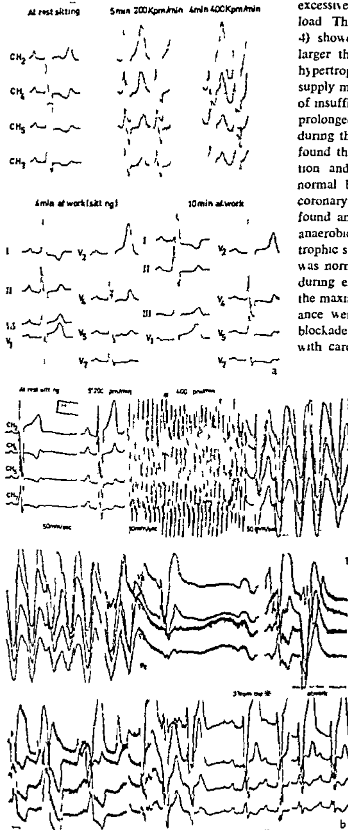
During the following months the patient suffered from severe chest pains and bouts of tachycardia and dyspnoea Medication with alprenolol (Aptin®) had a positive effect on the symptoms but in view of the complications seen with propranolol this therapy was interrupted At the last control about ten months later an exercise test was performed on somewhat lower work loads (Table I) The resting ECG was unchanged and no remarkable arrhythmia occurred during the work test. The heart volume was normal 440 ml/sq m body surface area The dynamic spirometry showed improved values compared with preoperatively

According to the last information from the patient in June 1969 the chest pains had disappeared and she was able to work whole time as a clerk

## DISCUSSION

It does not seem probable that increasing stenosis or operative sequelae were the cause of the arrhythmia The operation was performed by an experienced surgeon During the $2^1/_2$ postoperative years there had been no complications and the auscultatory findings were unchanged The cardiac complication was hardly a random incident as the patient s physical working capacity had been tested several times and had been unchanged since the preoperative test showing a high and constant heart rate during the highest work load but no arrhythmia As the patient had no other medication it seems likely that propranolol played a causal role in the cardiac complication

The negative chronotropic effect of propranolol was seen as bradycardia at rest and lesser heart rate increase at work especially at 200 kpm/min There were no signs of the negative dromotropic effect at rest as the PQ time was shorter despite the bradycardia and the QRS duration was unchanged but at 200 kpm/min the PQ time slightly increased As the arrhythmia occurred during exercise it is probable that the reason for the attack was insufficient cardiac oxygen supply and/or




excessive sympathetic activity at the higher work load Though the preoperative angiography (Fig 4) showed wide coronary arteries suggesting a larger than normal coronary blood flow to the hypertrophied myocardium the cardiac oxygen supply may be suspected to have been at the limit of insufficiency at work as the QRS duration was prolonged to 12 csec at the highest work load during the exercise test in 1966 Gorlin et al (6) found the relation b tween the oxyg n consumption and the hypertrophic muscle mass to be normal but others (1) despite a larger than normal coronary blood flow ($^{133}$Xe and N_O technique) found an insufficient cardiac oxygen supply and anaerobic metabolism in a patient with hyper trophic subaortic stenosis The cardiac metabolism was normalized by pronethanolol at rest but not during exercise According to Epstein et al (5) the maximal physical working capacity and endur ance were decreased during acute b ta receptor blockade both in normal subjects and in patients with cardiac diseases as the negative ionotropic

*Fig 4* The ECG reaction at the exercise test, performed sitting on an electrically braked bicycle ergometer with wo k loads ̲00 and 400 kpm/min. *(a)* No medication, chest pains occurred *(b)* After three days of propranolol treatment, no subjective symptoms until after bout 4 min at 400 kpm min when entricular tachycardia oc curred, short bouts of ventricular fibrillation between short periods of asystole and periods with hete otopic ventricular beats, then return to sinus rhythm about 3 min from the beginning of the arrhythmia

effect reduced the cardiac output which at sub maximal but not maximal work loads was completely compensated for by an increased peripheral oxygen uptake and an increased arteriovenous oxygen difference In our patient the high heart rate increase from 89 beats/min at 200 kpm/min to 134 beats/min after 2 min and 154 beats/min after 4 min 400 kpm/min, might have been a compensatory mechanism to answer the peripheral oxygen demands especially as the stroke volume probably cannot be maintained in hypertrophic subaortic stenosis because of decreased compliance of the left ventricle However the degree of stenosis might have decreased with increasing heart rate (7) if the sympathetic activity did not cause excessive contractility The tendency to airway obstruction seen from the PEF values during propranolol treatment in our patient should not have influenced the oxygenation significantly According to the literature ventricular arrhythmias may be caused by increased sympathetic stimulation on localized ischaemic myocardial areas with changes in potassium balance (8) or changes in aortic pressure (9) Pearse (13) found increased noradrenaline content of the myocardium in some patients with hypertrophic subaortic stenosis

In the absence of haemodynamic studies a definite cause for the arrhythmia cannot be stated We report this hazardous complication to point out that beta receptor blockade in hypertrophic subaortic stenosis may not only be ineffective (4) but even harmful at least during exercise Complications may even occur during subjective improvement and without any warning symptoms

## REFERENCES


1 Brink, A J Lewis C M & van Heerden P D R. Coronary blood flow and myocardial metabolism in obstructive cardiomyopathy Observations before and after treatment with beta adrenergic blocking agent Amer J Cardiol 19 548 1967

2. Cherian G., Brockington, I F Shah P M Oakley C M & Goodwin J F Beta adrenergic blockade in hypertrophic obstructive cardiomyopathy Brit. med J 1 895 1966

3 Cohen, L. S & Braunwald, E. Amelioration of angina pectoris in idiopathic hypertrophic subaortic stenosis with beta adrenergic blockade Circulation 35 847 1967

4 Editorial Hypertrophic obstructive cardiomyopathy Brit. med. J 2 5478 1966

5 Epstein, S E., Robinson B F Kohler R. L & Braunwald, E Effects of beta adrenergic blockade on the cardiac response to maximal and submaximal exercise in man J clin. Invest. 44 1745 1965

6 Gorlin R Cohen L. S Elliott, W C., Klein M D & Lane F J Haemodynamics of muscular subaortic stenosis (obstructive cardiomyopathy) In Ciba Foundation Symposium Cardiomyopathies (ed. G E W Wolstenholme and M O Connor) p 76 Churchill London 1964

7 Hancock E. & Eldridge F Muscular subaortic stenosis Reversibility with varying cardiac cycle length Amer J Cardiol 18 515 1966

8 Harris A S Genesis of ventricular tachycardia and fibrillation following coronary occlusion In Mechanism and therapy of cardiac arrhythmias The Fourteenth Hahnemann Symposium (ed L. Dreifus & W Likoff) Grune & Stratton, New York 1965

9 Harris A. S Otsuma H & Boggs P B Changes in infarction arrhythmias resulting from changes in arterial pressure Amer J Physiol 210 526 1966

10 Harrison D C Braunwald E. Glick G Mason, D T Chidsey C A. & Ross J Effects of beta adrenergic blockade on circulation with particular reference to observations in patients with hypertrophic aortic stenosis Circulation 29 84 1964

11 Leachmann R D & Leathermann, L. L Idiopathic hypertrophic subaortic stenosis Cardiovasc res Bull. 7 9 1968

12 Muscholl E & Rahn K. H Adrenerge alfa and beta receptoren und ihre spezifischen Hemmstoffe Klin Wschr 46 113 1968

13 Pearse A G E. The histochemistry and electron microscopy of obstructive cardiomyopathy p 132. In Ciba Foundation Symposium Cardiomyopathies (ed G E W Wolstenholme and M O Connor) p 132. Churchill, London 1964

14 Stephen S A Unwanted effects of propranolol Amer J Cardiol 18 463 1966

15 Wolfsson, S Discussion Symposium over beta receptor blocking agents Amer J Cardiol 18 470 1966.

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# TREATMENT OF THYROIDITIS

## *Observations from 207 Cases Collected during the Period 1960–1968*

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*Abstract* During the period 1960–1968 207 cases of thyroiditis were observed in the Second Department of Surgery of Sahlgrenska sjukhuset All patients were continuously followed up Subacute thyroid tis was seen in ?9 cases, and focal thyroiditis together with other thyroid diseases in 0 cases Of 178 patients with chronic thyroiditis, 47 were primarily operated upon 130 patients were treated with thyroid hormone and one patient was not treated Eleven patients were operated upon later because of unsatisfactory response to the hormone treatment. This paper presents an account of the results of treatment. The principles of treatment of chronic thyroiditis are discussed

During recent years inflammatory diseases of the thyroid have attracted an ever increasing interest (7) This is due partly to the discovery of antibodies against thyroid antigen which can cause thyroiditis (10) and partly to the possibility of verifying the diagnosis thyroiditis with fine needle as well as coarse needle biopsy (6 9) Increasing numbers of patients with thyroiditis therefore come for treatment A number of questions present themselves in connection with the choice of m thod of treatment

Does the patient with thyroiditis on the whole need to be treated even if he is euthyroid and lacks local mechanical symptoms of his goiter?

If one chooses treatment with thyroid hormone must this treatment then be carried on as a life long substitution therapy?

How effective is treatment with thyroid hormone?

Which patients should be operated on?

Which operation method should be used in chronic thyroiditis?

What are the results of surgical treatment?

## MATERIAL

The material consists of all cases of thyroiditis observed during the period 1960–1968 at our Thyroid Unit in Department of Surgery II Sahlgrenska Hospital During this period our clinic was visited by a total of 1772 thyroid patients. Of these patients 207 (~12 ) had thyroiditis There were 29 cases of subacute and 178 cases of chron thyroiditis, 20 of whom had focal and 158 general chronic thyroiditis.

The composition of the material regarding diagnosis, age and sex is shown in Table I

The sex distribution for patients with chronic thyroiditis is one man to nine women and for patients with subacute thyroiditis one man to five women.

The material includes 11 patients with a recurrence of go ter Four patients with thyroiditis were suffering from parathyroid disease In two of these cases the diagnosis hyperparathyroidism was made before the operation and in two a parathyroid adenoma was found in connection with exploration of the thyroid.

Table II shows the observation time in years. It will be seen from the table that 15 patients had been diagnosed as thyroiditis before our thyroid clinic was organised in 1960 All these patients had been operated upon earlier and the diagnosis thyroiditis had been made in connection therewith These patients were treated post-operatively in our thyroid clin c

Nearly half of the material had been observed for fi e years or more In a small number of patients the observation time was less than half a year and they have not been taken into account in judging the results of treatment, as is evident from Table VI

## METHODS

### *Diagnostic methods*

In patients with subacute thyroiditis the diagnosis in general could be made on the basis of the typ cal clinical findings. In 11 of the 29 patients, howe er the diagnosis was also confirmed by fine needle b opsy Of 178 patients with chronic thyroiditis the d agnosis was made on clinical grounds only in six cases. In all the other patients the

Table I *All patients with thyroiditis classified according to diagnosis age and sex*

| Age group (y) | Subacute thyroiditis | | Focal thyroiditis | | Chronic thyroiditis | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | ♂ | ♀ | ♂ | ♀ | o | ♀ | ♂ | ♀ | |
| 10–19 | 0 | 1 | 0 | 0 | 1 | 5 | 1 | 6 | 7 |
| 20–29 | 0 | 1 | 0 | 0 | 0 | 11 | 0 | 12 | 12 |
| 30–39 | 2 | 9 | 0 | 3 | 2 | 24 | 4 | 36 | 40 |
| 40–49 | 2 | 6 | 0 | 6 | 4 | 39 | 6 | 51 | 57 |
| 50–59 | 1 | 5 | 0 | 6 | 3 | 35 | 4 | 46 | 50 |
| 60–69 | 0 | 2 | 0 | 3 | 4 | 24 | 4 | 29 | 33 |
| 70–79 | 0 | 0 | 0 | 2 | 1 | 3 | 1 | 5 | 6 |
| 80–89 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 2 |
| Total | 5 | 24 | 0 | 20 | 15 | 143 | 20 | 187 | 207 |

diagnosis was based on cytological or histological investigations as shown in Tables III and IV The diagnosis was thus based chiefly on cytological and patho-anatomical diagnosis.

All patients were investigated with reference to their thyroid function (PBI, resin triiodothyronine test, radioiodine test, BMR) and in a large number of cases estimation of thyroid antibodies in the serum was also carried out. The result of these investigations has partly been given in another connection (5 9) As the cytological or histological diagnosis is that on which one can base a reliable and objective classification, only this parameter has been used here.

### *Principles of therapy*

subacute thyroiditis generally no treatment has been en. In exceptional cases with pronounced subjective in the initial stage (such as tenderness, fever and mechanical local symptoms) anti-inflammatory treatment (corticosteroids, phenylbutazone oxyphenbutazone) has been used with success. A number of patients have during a shorter period been treated with thyroid hormone until their goiter diminished or disappeared. Of the 29 patients with subacute thyroiditis, consequently 12 patients needed to receive thyroid hormone treatment. In seven of the patients there was goiter before the occurrence of subacute thyroiditis, and treatment with thyroid hormone continued in these cases also after the subacute thyroiditis had disappeared.

Of the 178 patients with chronic thyroiditis 177 were treated. Forty-seven patients were operated on primarily while 130 patients were primarily treated with thyroid hormone (Fig. 1 and Table V) In 11 cases a secondary operation was performed because the thyroid hormone treatment did not lead to a decrease of the goiter

Fig. 2 shows the age distribution of all the 58 operated and 120 non-operated patients with chronic thyroiditis. As will be seen from this figure the age distribution in both groups is on the whole similar

The operation methods will be seen from Table VI. In 25 cases a bilateral and in 29 cases a unilateral, operation was carried out.

## RESULTS

### *A. Unoperated Cases*

Of 29 patients with subacute thyroiditis there were seven cases with a goiter remaining after the

Table II *Observation time in 207 cases of thyroiditis*

| Diagnosis (y) | Subacute thyroiditis | Focal thyroiditis | Chronic thyroiditis | Observation time: No of cases/y | Total no of cases | Years |
|---|---|---|---|---|---|---|
| 1959 and earlier | 1 | 2 | 12 | 15 | | >9 |
| 1960 | | | 15 | 15 | 30 | 9 |
| 1961 | | | 6 | 6 | 36 | 8 |
| 1962 | 1 | | 16 | 17 | 53 | 7 |
| 1963 | 3 | | 12 | 15 | 68 | 6 |
| 1964 | 4 | 3 | 21 | 28 | 96 | 5 |
| 1965 | 1 | 4 | 19 | 24 | 120 | 4 |
| 1966 | 3 | 3 | 9 | 15 | 135 | 3 |
| 1967 | 9 | 4 | 21 | 34 | 169 | 2 |
| 1968 | 7 | 4 | 27 | 38 | 207 | 1 |
| Total | 29 | 20 | 158 | 207 | | |

symptoms of thyroiditis had disappeared This goiter was treated continuously with thyroid hormone In 22 patients there was a total regression of the disease

In 120 patients with chronic thyroiditis the observation time was for eight patients less than 6 months therefore these cannot yet be judged There remain 112 patients No change of status took place in 14 patients (13 ) *In 87% of all patients treated with thyroid hormone a regression of the goiter was observed* In 20% of 101 patients with diffuse chronic thyroiditis the goiter and all subjective symptoms of the disease disappeared (Table VII)

The total number of patients treated with thyroid hormone pre- and/or post-operatively was 155 In these side effects were observed in 12 cases (8%) *Thyrotoxicosis was observed in six* cases One of these patients was operated upon In the other five the thyrotoxicosis could be

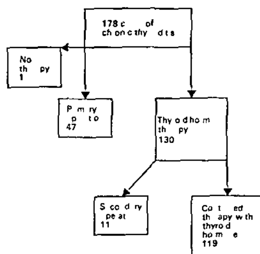



*Fig 1* Principles of treatment

treated with antithyroid drugs In six patients light cardiovascular symptoms appeared which however in no case led to a cessation of the thyroid hormone treatment. After a slight reduction of dose the symptoms disappeared No other side effects of the *l* thyroxin preparation (Levaxin®) could be observed

Table III *Diagnostic methods*

| Method | No of cases |
|---|---|
| Fine needle biopsy cytology | 117 |
| Coarse needle biopsy histology | 48 |
| Postoperative pathoanatomical diagnosis | 57 |
| Clinical d agnosis only | 24 |

Table IV *All patients with thyroiditis classified according to diagnosis and diagnostic method used*

| | No of patients with thyroiditis | | | |
|---|---|---|---|---|
| Diagnostic method | Sub acute | Focal | Chronic | Total |
| Only clinical d agnos s | 18 | | 6 | 24 |
| Fine needle b opsy cytology | 11 | 9 | 64 | 84 |
| Coarse needle biopsy histology | | 2 | 13 | 15 |
| Postoperative diagnosis only | | 5 | 42 | 47 |
| F ne and coarse needle biopsy | | 2 | 25 | 27 |
| Fine needle biopsy and postoperative diagnosis | | 1 | 5 | 6 |
| Coarse needle biopsy and postoperative diagnosis | | 1 | 3 | 4 |
| Total | 9 | 0 | 158 | 207 |

The diagnos s based on cytology and/or histology in 183 out of 207 cases w th thyroiditis (=88 of the material) In the ser es with chronic thyroid tis the d agnos s based on cytology and/or histology in 96 of all cases

## B *Operated Patients*

Altogether 58 patients have been operated upon Forty seven were operated on without preliminary thyroid hormone treatment 11 because the thyroid hormone treatment seemed to be ineffective

The indications for surgery are shown in Table VIII *In the main the indication for primary* operation was the mechanical symptoms of the goiter with compression of the trachea In eight cases the operation was done on a suspicion of malignancy In six cases the patients had thyro-

Table V *All patients with thyroiditis classified according to diagnosis and therapy*

| | No of pat ents w th thyroiditis | | | |
|---|---|---|---|---|
| Therapy | Sub-acute | Focal | Chronic | Total |
| No therapy | 17 | | 1 | 18 |
| Thyroid hormone | 12 | 13 | 106 | 131 |
| Surgery and postoperative thyroid hormone | | 7 | 51 | 58 |
| Total | 29 | .0 | 158 | 07 |

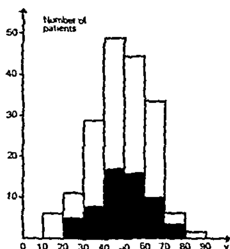



*Fig 2* Operated and non-operated patients with chronic thyroiditis according to age ■ number of operated cases. □ number of non-operated cases.

toxicosis In two cases the pre-operative investigation had indicated that there was a cyst in the thyroid, and biopsy had not given support for the diagnosis of thyroiditis The indication was in these cases the mechanical and local symptoms which were caused by the thyroid cyst. Of 11 patients with a recurrent goiter two were operated on In these too the mechanical symptoms were the indication for operation

Eleven patients were operated on because the thyroid hormone treatment was ineffective Often there was an adequate primary response to thyroid hormone treatment, but the goiter began to grow again and the symptoms again increased, therefore operation was indicated Of these patients four had been treated with thyroid hormone for 6–12 months one for 13 months three between 25 and 36 months three patients for 4 5 and 6 years respectively before operation was necessary In none of these patients was any malignant disease found in the post-operative examination of the removed thyroid tissue

Table IX shows the distribution of the operated patients during the period Fifteen of the patients had been operated on before 1960 and 43 after 1960 In about half of the material the observation period is six years or longer

The post-operative examination of the resected thyroid material showed that in 23 of the 58 operated cases there was thyroiditis simultaneously with other thyroid disease In 13 cases there

Table VI *Type of operation*

| Type of operation | No of operations |
|---|---|
| Total thyroidectomy | 4 |
| Subtotal bilateral thyroidectomy | 16 |
| Total and subtotal hemithyroidectomy | 5 |
| All bilateral operations | 25 |
| Total hemithyroidectomy | 20 |
| Subtotal hemithyroidectomy | 9 |
| All unilateral operations | 29 |
| Incomplete description of surgical procedure | 4 |
| Total number of operations | 58 |

Table VII *All non-operated patients with thyroiditis classified according to diagnosis and result of treatment*

| Results | No of patients with thyroiditis | | | |
|---|---|---|---|---|
| | Sub-acute | Focal | Chronic | Total |
| Total regression of goiter and symptoms | 22 | | 20 | 42 |
| Regression of size of goiter | 7 | 7 | 71 | 85 |
| No change of status | | 4 | 10 | 14 |
| Progression of disease | | | | 0 |
| Time of observation too short | | 2 | 6 | 8 |
| Total | 29 | 13 | 107 | 149 |

In 101 cases of unoperated diffuse chronic thyroiditis with an observation time of more than 6 months a regression of goiter observed in 90 of the cases. In 40 % of the cases the goiter disappeared totally

Table VIII *All operated patients with thyroiditis classified according to indication for surgery*

| Indication | No of pats. operated on |
|---|---|
| Mechanical symptoms of goiter | 27 |
| Suspected malignancy | 8 |
| Thyrotoxicosis | 6 |
| Hyperparathyroidism | 2 |
| Thyroid cyst | 2 |
| Recurrent goiter | 2 |
| Total primary operations | 47 |
| Secondary operations (inadequate response to therapy with thyroid hormone) | 11 |
| Total patients operated on | 58 |

was nodular non toxic goiter in four a solitary thyroid adenoma in one a solitary thyroid cyst, in four cases diffuse toxic goiter and in one case nodular toxic goiter (Table X) In 54 cases the diagnosis was chronic lymphoid thyroiditis and in four cases chronic fibrous thyroiditis in two of which nearly a Riedel s goiter

In all operated cases thyroid hormone was given post-operatively The dose of *l* thyroxin given in mg will be seen from Table XI In about

Table IX *Series of operated patients classified according to year of operation and observation time*

| Operation (y) | No of operations | Observation time: Years | Total no of pats |
|---|---|---|---|
| 1959 and earlier | 15 | >9 | 15 |
| 1960 | 7 | 9 | 22 |
| 1961 | 3 | 8 | 25 |
| 1962 | 3 | 7 | 28 |
| 1963 | | 6 | 28 |
| 1964 | 10 | 5 | 38 |
| 1965 | 1 | 4 | 39 |
| 1966 | 3 | 3 | 42 |
| 1967 | 12 | 2 | 54 |
| 1968 | 4 | 1 | 58 |

Table X. *All patients with chronic thyroiditis operated on classified according to pathoanatomical diagnosis*

| Pathoanatomical diagnosis | Diagnostic group: Focal thyroiditis | Chronic thyroiditis | Total |
|---|---|---|---|
| Diffuse lymphoid thyroiditis | 1 | 30 | 31 |
| Nodular non toxic goiter and diffuse lymphoid thyroiditis | 4 | 9 | 13 |
| Adenoma of the thyroid and diffuse lymphoid thyroiditis | 2 | 2 | 4 |
| Thyroid cyst and diffuse lymphoid thyroiditis | | 1 | 1 |
| Diffuse toxic goiter and diffuse lymphoid thyroiditis | | 4 | 4 |
| Nodular toxic goiter and diffuse lymphoid thyroiditis | | 1 | 1 |
| Chronic fibrous thyroiditis | 2 | 2 | 4 |
| Total | 9 | 49 | 58 |

In 23 of 58 operated cases (40 %) thyroiditis occurred simultaneously with another thyroid disease.

Table XI *Postoperative therapy with thyroid hormone*

| Average dose of *l* thyroxin (mg) | No of pats. |
|---|---|
| 0 10 | 7 |
| 0 15 | 24 |
| 0 20 | 20 |
| 0 30 | 3 |
| Incomplete information | 4 |
| Total | 58 |

Approximately 80 of all operated patients treated post operatively with a dose of 0 15–0 20 mg of *l* thyroxin

Table XII *Postoperative complications*

Total no of operations 58

| Complication | No of cases: Unilateral operation | Bilateral operation | Total | All operated cases ( ) |
|---|---|---|---|---|
| Permanent unilateral vocal cord paralysis | 2 | 0 | 2 | 3 4 |
| Hypoparathyroidism | 0 | 2 | 2 | 3 4 |
| Postoperative growth of goiter | 8 | 1 | 9 | 15 5 |

80 % of the cases a substitution dose of 0 15–0 20 mg *l* thyroxin was used.

The post operative complications are shown in Table XII One case of lasting *vocal cord paralysis* occurred in the operation of a recurrent goiter In 56 primary operations there was one case of lasting vocal cord paralysis (1 8% of cases)

One case of *post-operative hypoparathyroidism* occurred in a patient who was operated on for hyperparathyroidism with simultaneous thyroiditis As four cases of parathyroid disease were diagnosed in the material 54 cases of thyroiditis were operated on without parathyroid disease being present at the same time After these 54 operations hypoparathyroidism appeared in one case i e in 1 9 %

*Post-operative growth* of goiter was registered in spite of thyroid hormone treatment in nine patients of whom five were operated on in 1959 or earlier In our own operations (1960 or later) recurrent goiter was observed in four patients, i e in 9 %

All of these four recurrences occurred after unilateral operations i e in 19 % of those operated upon on one side only

## DISCUSSION

The treatment of thyroiditis has during recent years been discussed by several authors (1 2 5 8 11)

Subacute thyroiditis may be dealt with on three principles no therapy thyroid hormone substitution and treatment with anti inflammatory agents The majority of cases go spontaneously to complete regression and demand no therapy Intensive cases need palliative treatment, anti inflammatory drugs often being of value Only cases with remaining goiter and signs of thyroid hypofunction need substitution with thyroid hormone Subacute thyroiditis may arise in an earlier existing goiter Thyroid hormone is then given to prevent the growth of existing goiter

The continued discussion is devoted to chronic thyroiditis which constitutes the major part of the material and also the greater therapeutic problem.

In chronic thyroiditis several methods of treatment may be chosen

(*a*) no therapy only continued observation

(*b*) treatment with thyroid hormone with the intention of reducing TSH stimulation of the thyroid, reducing or preventing growth of the goiter and hindering the development of hypothyreosis

(*c*) treatment with corticosteroids or other anti inflammatory agents

(*d*) specific treatment of hypo- or hyperthyreosis (thyroid blocking agents and/or thyroid hormone)

(*e*) operation.

Chronic thyroiditis is a progressive disease which may lead to total destruction of the follicle epithelium in the thyroid and cause permanent hypothyroidism (2 7 9) The question posed in the introduction whether patients with chronic thyroiditis need to be treated must therefore be answered unconditionally in the affirmative

Treatment with thyroid hormone does not affect the immunological processes which maintain the progress of the disease Nonetheless this treatment may stop the progress of the disease lead to a reduction of the goiter and prevent hypothyroidism. During the observation period, which in half of the cases exceeded five years this treatment in fact proved effective in 90 of cases In the light of the fact that diffuse chronic thyroiditis is a progressive and chronic disease these patients should be treated with thyroid hormone for the rest of their life The treatment should be regarded as a hormone substitution

Which patients should be operated on? We operated on all patients in whom thyroid hormone treatment did not give the desired result (11 cases) and on patients in whom there were indications for surgery from the start. One of these indications for surgery is suspected malignancy This problem has been discussed earlier by among others Persson (9) who gave an account of two cases of malignant thyroid disease in his material of thyroiditis based on cytodiagnosis In none of the patients in our material has a clinical suspicion of malignancy arisen in the course of the years Our principle is to treat patients with chronic thyroiditis primarily with thyroid hormone If we do not get a satisfactory response to this treatment we operate among other reasons, in order to exclude malignant thyroid disease None of the 11 patients operated upon on these indications had malignant thyroid disease In two of the patients there was clinically a solitary thyroid tumor and the patients were operated upon on that indication These adenomata were benign however When judging a patient with thyroiditis one should pay regard to the clinical signs of malignancy We always carry out an examination which besides an investigation of thyroid function includes X ray of the soft parts of the neck fine or coarse-needle biopsy and in some cases angiography of the arteries of the thyroid (12)

Primary operation should in my opinion be carried out on the following indications

(*a*) suspected malignancy (investigation as above)

(*b*) pronounced mechanical symptoms with tracheal compression (X ray)

(*c*) other simultaneously present thyroid or parathyroid disease which in itself constitutes an indication for surgery

Chronic thyroiditis is a general disease of the thyroid, and one should therefore carry out a bilateral operation. The result of our follow up

shows that the frequ ncy of recurrent goiter after unilateral operations is relatively high This fre quency seems to increase with observation time

Total thyroidectomy is not indicated and is attended by some major post-operative complica tions One case of existing hypoparathyroidism was observed after total thyroidectomy Another case was observed in a patient who was operated on both for parathyroid disease and thyroiditis If one excludes patients with simultaneous para thyroid disease and all the patients who were operated on by total thyroidectomy not a single case of lasting vocal cord paralysis or lasting hypoparathyroidism occurred in the patients with thyroiditis who underwent a bilateral operation

*The result of surgical treatment is thus satis* factory if a bilateral subtotal resection is carried out and if the patient post-operatively receives adequate substitution generally 0 15–0 20 mg of *l* thyroxin daily

Our observations of four patients with simul taneous chronic thyroiditis and parathyroid disease are of special interest These cases are report ed and discussed in another context (3 4) How ever this coincidence of thyroiditis and parathyroid disease deserves to be pointed out Parathyroid disease was present at the same time in four out of 58 patients who were operated on for chronic thyroiditis (6 9 ) We earlier found (3 4) five cases of chronic thyroiditis in a material of 160 patients operated on for hyperparathyroidism (3 1 %)

## REFERENCES


1 Astwood, E B Management of thyroid disorder J Amer med Ass 186 585 1963
2 Heimann, P Pathophysiological aspects on surgical treatment of thyroid disease Progr Surg (Basel) 7 1 1969
3 Heimann P Nilsson O Mortensen A. & Hansson G Parathyroid and thyroid disease I Acta chir scand In press
4 Heimann P Nilsson O & Hansson G Parathyroid and thyroid disease II Acta chir scand In press
5 Heimann P Persson P S & Risholm L Clinical observations in patients with diffuse lymphoid thyroid itis with special reference to the effect of treatment with thyroid hormone Acta med scand Suppl. 483 1968
6 Heimann P & Schnurer L B Needle biopsy of the thyroid gland A report of 117 cases with special reference to the diagnostic accuracy of the method in benign thyroid disorders. Acta chir scand. 128 85 1964
7 Masi A. T Hashimoto s disease An epidemiological *study based on a community wide hospital survey* J chron Dis 18 35 1965
8 McConahey W M Woolner L. B Black, B M & Keating, F R. Jr Effect of desiccated thyroid in lymphocytic (Hashimoto s) thyroiditis. J clin Endocr 19 45 1959
9 Persson P S Cytodiagnosis of thyroiditis. A comparative study of cytological histological immunological and clinical findings in thyroiditis particularly in diffuse lymphoid thyroiditis (in collaboration with Biberfeld, G Heimann P Jonsson J Risholm L. & Schnurer L B) Acta med scand Suppl 483 1968
10 Roitt, I M Doniach D Campbell P N & Hudson R. V Autoantibodies in Hashimoto s disease (lymphadenoid goitre) Lancet 2 8 0 1956
11 Skillern, P G Treatment of Hashimoto s disease and invasive thyroiditis. In Modern treatment (ed. E. L. Becker & E A Carr Jr) p 159 Harper & Row New York 1964
12. Wickbom, I Zachrisson B F & Heimann P Thyroid angiography Acta radiol (Stockh) 6 497 1967

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# VARIATIONS IN PLASMA GLUCOSE IN NORMAL SUBJECTS AND DIABETICS IN THE FASTING STATE

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*Abstract* In 23 normal subjects and seven juvenile diabetics the plasma level of glucose has been determined at 30-sec intervals under basal conditions In seven of the normal persons venous blood was studied in the remaining subjects arterial blood. Only arterial blood was studied in the diabetic group In some but not all of the subjects the variations in the glucose level significantly exceeded the error of the measurement. There were short lasting, irregular undulations in the gluose concentration of an amplitude of 2–8 mg/100 ml during periods of 2–6 min. There was no significant difference in glucose variations between normals and diabetics This may be interpreted as evidence that an intact beta-cell function is not necessary for maintaining a normal glucose homeostasis. The authors advance the hypothesis that the glucose concentration in the fasting state is regulated primarily by a feedback mechanism between glucose and the activities of the enzymes involved in the carbohydrate metabolism of the liver This mechanism does not require normally functioning endocrine glands, only the presence of certain minimum concentrations of the glucostatic hormones in particular insulin and possibly glucagon.

It is well known that the human body in the fasting state maintains its blood glucose level with surprising constancy However it is a disputed point how much the concentration varies around the mean from minute to minute (9) One of us (9) has previously demonstrated that the glucose level in venous whole blood under basal conditions varies rhythmically in normals with undulations of 3–4 min and amplitudes of 2–6 mg/100 ml

The object of the present study was to investigate whether changes in plasma water concentration or peripheral utilization have contributed to the variations found in the glucose concentration in venous blood In addition the findings in diabetics and in normals were compared to elucidate whether a normal beta-cell function in the pancreas is a presupposition for maintaining the fasting glucose concentration

## MATERIAL AND METHODS

The material comprised 23 normal weight non-diabetics (the normal series) and seven normal weight juvenile diabetics who required insulin All the diabetics had been on long acting insulin for at least four years. The last injection was administered 14–24 h before the investigation. In seven of the normal subjects variations in the venous plasma level of glucose were studied while arterial plasma was studied in the others. Table I gives the age sex, diagnosis, duration of diabetes, and insulin requirement

The subjects were ambulatory patients or members of the hospital staff After about 14 h fasting the subjects were made to lie down in a quiet room. For investigation of venous plasma a needle (Mediplast) was inserted into an antecubital vein after a few min rest This was followed by a preliminary period of 10–20 min to accustom the subjects to the experimental conditions and thereby to avoid as far as possible disturbing impulses from the autonomic nervous system During the preliminary period the needle was kept patent by slow infusion of an isotonic saline solution Thereupon the infusion was stopped, 10 ml blood was aspirated and discarded, and at 30-sec intervals 2 ml blood was aspirated in a few seconds into a 2 ml syringe whose contents were immediately emptied into a tube in an icebath to reduce glycolysis The blood was drawn without application of a tourniquet

To investigate arterial plasma we introduced after infiltrated anaesthesia, a needle into the femoral artery The needle was connected to a 5–10 cm long plastic tube and the tube and needle were flushed with a solution of heparin saline (1 000 I U heparin ml 0 9% NaCl) The system was closed with Pean s forceps Samples were drawn at 30-sec intervals by opening the forceps and letting the blood pulsate through the needle and tube The first 2 ml of each sample were discarded in order not to include blood which had been stagnant in the system between the samplings. The removal of the samples took less than 5 sec and the entire experimental period lasted for from 6–15 min. The procedure was entirely painless in the majority of cases

The samples were examined on the same day or frozen.

Plasma glucose was determined after protein precipitation by a *p*-bromaniline method (8) which owing to its simplicity permits greater precision than the conventional

*Table I Variations in glucose concentration with ($s_1$) and without ($s$) correction for the slope of the regression line ($b$)*

| Case no | Diagnosis | Age | Sex | Duration of diabetes (y) | Insulin requirement /24 h U | No of blood samples | $y$ (mg/100 ml) | S E |
|---|---|---|---|---|---|---|---|---|
| *Venous plasma* | | | | | | | | |
| 1 | Treated myxoedema | 36 | ♀ | | | 30 | 78 2 | 1 76 |
| 2 | Healthy | 26 | ♂ | | | 30 | 92 0 | 1 48 |
| 3 | Duodenal ulcer | 40 | o | | | 30 | 104 1 | 2 71 |
| 4 | Healthy | 22 | ♀ | | | 30 | 85 1 | 1 83 |
| 5 | Urticaria | 33 | ♂ | | | 29 | 92 7 | 1 30 |
| 6 | Healthy | 20 | ♀ | | | 30 | 86 7 | 1 97 |
| 7 | Pneumonia | 16 | ♂ | | | 30 | 96 7 | 2 04 |
| Mean | | | | | | | | 1 87 |
| *Arterial plasma* | | | | | | | | |
| 8 | Duodenal ulcer | 35 | ♂ | | | 18 | 90 7 | 1 52 |
| 9 | Hypernephroma | 50 | o | | | 22 | 101 5 | 0 5 |
| 10 | Art scl heart dis | 62 | ♀ | | | 30 | 104 2 | 1 20 |
| 11 | Chr colitis | 50 | ♀ | | | 24 | 82 7 | 0 80 |
| 12 | Chr colitis | 38 | ♀ | | | 24 | 97 1 | 63 |
| 13 | Pneumonia | 19 | ♂ | | | 12 | 85 8 | 2 40 |
| 14 | Dyspepsia | 31 | ♀ | | | 28 | 93 6 | 2 53 |
| 15 | Cerebr art scl | 50 | o | | | 13 | 95 5 | 1 54 |
| 16 | Collagenosis | 60 | ♂ | | | 25 | 76 2 | 1 81 |
| 17 | Chr colitis | 75 | ♂ | | | 25 | 89 6 | 1 50 |
| 18 | Chr colitis | 52 | ♀ | | | 30 | 90 9 | 1 92 |
| 19 | Cephalalgia | 44 | ♀ | | | 15 | 87 7 | 1 54 |
| 20 | Intellectual impairment | 56 | ♀ | | | 30 | 92 7 | 1 73 |
| 21 | Chr colitis | 64 | ♂ | | | 25 | 82 5 | 1 00 |
| 22 | Chr pyelonephritis | 40 | ♀ | | | 17 | 87 1 | 1 0 |
| 23 | Varices of the legs | 73 | ♀ | | | 25 | 88 8 | 1 52 |
| an | | | | | | | | 1.55 |
| | Diabetes mellitus | 33 | ♂ | 22 | 34 | 25 | 134 2 | 1 30 |
| | Diabetes mellitus | 20 | ♀ | 11 | 28 | 24 | 228 7 | 1 18 |
| 26 | Diabetes mellitus | 22 | ♀ | 4 | 36 | 17 | 256 5 | 3 9 |
| 27 | Diabetes mellitus | 21 | ♂ | 13 | 32 | 30 | 318 3 | 4 16 |
| 28 | Diabetes mellitus | 43 | o | 4 | 20 | 13 | 118 0 | 1 69 |
| 29 | Diabetes mellitus | 69 | ♀ | 11 | 60 | 30 | 119 4 | 3 43 |
| 30 | Diabetes mellitus | 21 | o | 8 | 34 | 19 | 266 8 | 2 62 |
| Mean | | | | | | | | 2.61 |

$y$ = the average glucose concentration in one experiment S E = standard error of the mean of the duplicate determinations $p$ and $p_1$ = significance level for $s$ and $s_1$ in relation to S E calculated on the basis of the $F$ distribution according to the formula $F = s^2/S E$ and $F = s_1^2/S E^2$ Thus $p$ and $p_1$ indicate whether the variations in glucose concentration in the individual experiments exceeded the error of measurement $b \pm s_b \times t$ = regression coefficient with appurtenant confidence interval ($p = 0 05$)

methods. All determinations were in duplicate During the glucose analyses proper the samples were randomized, the person who performed the glucose analyses being un aware of which samples made up a pair in the duplicate determination.

The standard error of the mean of the duplicate deter minations S.E., was calculated separately for each experi ment according to the formula S E = ± square root of sigma $D^2/2n$ where $D$ is the difference between the duplicate determinations and $n$ the number of duplicate determinations The variations in glucose level in an experiment are stated partly as the standard deviation ($s$) of the glucose values from the mean glucose concentration ($y$) in the experim nt, and partly as the standard devia tion ($s_1$) of the glucose values from the regression line between glucose concentration and time Both quantities were re ated to S E and the corresponding ratios between the variances were calculated giving a $p$ value for the ratios concerned.

To ascertain whether a decreasing or increasing tendency in gluocse level occurred during the entire experimental period, the regression coefficent $b$ of the linear regression between the glucose concentration and time was calculated by the method of least squares ($b$ = sigma $xy$/sigma $x^2$)

For confidence intervals and comparison of mean values Student s $t$ test was used

| | $p$ | $s_1$ | $p_1$ | $b \pm s_b \times t$ |
|---|---|---|---|---|
| 95 | <0 01 | 2 04 | >0 05 | +0 49±0 18 |
| 8 | <0 01 | 2 28 | <0 01 | −0 18±0 19 |
| 05 | <0 01 | 3 50 | >0 05 | −0 43±0 32 |
| 9 | >0 05 | 2 29 | >0 05 | −0 11±0 0 |
| .24 | <0 01 | 1 72 | >0 05 | +0 35±0 16 |
| 01 | >0 05 | 2 01 | >0 05 | −0 02±0 18 |
| 04 | <0 05 | 2 68 | >0 05 | +0 33±0 25 |
| 69 | | 3 36 | | |
| 84 | >0 05 | 0 94 | >0 05 | +0 59±0 19 |
| 95 | <0 01 | 1 95 | <0 01 | +0 23±0 .6 |
| 37 | <0 01 | 2 37 | <0 01 | +0 14±0 18 |
| 53 | <0 01 | 3 19 | <0 01 | −0 44±0 40 |
| 0 | <0 01 | 4 05 | <0 05 | +1 97±0 48 |
| 49 | <0 01 | 6 49 | <0 01 | +1 16±2 40 |
| 33 | >0 05 | 1 72 | >0 05 | −0 38±0 17 |
| 81 | >0 05 | 1 81 | >0 05 | +0 22±0 60 |
| 69 | >0 05 | 1 69 | >0 05 | −0 15±0 18 |
| 5 | <0 01 | 1 97 | >0 05 | −0 44±0 12 |
| 35 | >0 05 | 1 35 | >0 05 | +0 07±0 12 |
| 13 | >0 05 | 2 13 | >0 05 | −0 38±0 61 |
| .26 | >0 05 | 1 6 | >0 05 | +0 03±0 11 |
| 09 | <0 01 | 2 09 | <0 01 | −0 06±0 22 |
| 11 | <0 01 | 2 17 | <0 01 | +0 24±1 40 |
| 94 | >0 05 | 1 94 | >0 05 | +0 24±0 0 |
| 73 | | 2 32 | | |
| 08 | <0 01 | 1 84 | <0 01 | +0 27±0 17 |
| 55 | <0 01 | 2 42 | >0 05 | +2 25±0 66 |
| 07 | <0 01 | 5 30 | <0 05 | +1 70±1 23 |
| 17 | <0 01 | 9 17 | <0 01 | +0 39±0 79 |
| 29 | >0 05 | 2.29 | >0 05 | −0 02±0 79 |
| 9 | >0 05 | 2 25 | >0 05 | +0 61±0 20 |
| 85 | <0 01 | 5 85 | <0 01 | +0 10±0 11 |
| 64 | | 4 16 | | |

## RESULTS

The results are given in Table I It may be seen that in about half the experiments there were variations in the glucose concentration which differed significantly from the error of measurement It may be mentioned that the precision (S E.) was relatively low in experiments showing a high glucose level Fig 1 illustrates some representative curves It will be noted that the undulations in venous as well as arterial plasma glucose were irregular showing changing undulation periods and amplitudes The duration of each period varied from 2–8 min No significant difference was found in the magnitude of the undulations in glucose concentration between venous and arterial plasma or in arterial plasma between normals and diabetics In addition the results from a previous study (9) on the undulations of the glucose level in venous whole blood in normals were compared with those in venous plasma glucose in the normal subjects of the present study No significant differences were found There was however a correlation between the mean glucose concentration in the experiments (y) and $s$ S E. ($r = 0\ 40$ $p < 0\ 05$) In other words a high glucose level in an experiment was correlated to greater variations in the glucose level If this relationship was taken into account and the plasma glucose concentration in venous plasma and whole blood was corrected to a glucose level of 100 mg/100 ml a comparison of the variations in the glucose level in venous whole blood (9) with those in venous plasma showed that the variations in the glucose level in whole blood significantly exceeded those in plasma ($t = 2\ 57$ $p < 0\ 02$) A similar correction for the difference in the glucose level in comparing the variations in the level between arterial and venous plasma in diabetics and normals showed no significant differences

## DISCUSSION

The investigation confirmed that in the fasting state many people show slight rapid changes in blood glucose concentration On the other hand we could not confirm the previously demonstrated regularity in these undulations (9) In venous as well as in arterial plasma the undulations in the glucose level were utterly irregular and varying in amplitude The finding that the changes in venous whole blood exceeded those in plasma presumably indicates that changes in haematocrit have contributed to the occurrence of the changes in venous whole blood However measurements of the haematocrit have not been done

The absence of a difference between changes in the glucose levels in venous and arterial plasma militates against the possibility of changes in peripheral utilization contributing to the undulations in the venous glucose level

In addition to the short lasting variations around

5 Cahill G F., Asmore J., Renold, A. E & Hastings, A B Blood glucose and the liver Amer J Med. 26 ‿64 1959
6 Cahill G F Herrera, M. G Morgan, A. P., Soeldno J S Steinke J., Levy P L Reichard G A. & Kipnis, D M Hormone fuel interrelationships during fasting J clin Invest. 41 1751 1966
7 Cerasi, E & Luft, R. The plasma insulin response to glucos‿ infusion in healthy subjects and in diabetes me litus Ac‿a endocr (Kbh) 55 278 1967
8 Deckert, T Method for determining glucose in plasma cerebrospinal fluid, and urine by means of p bromaniline Scand J clin. Lab Invest. 20 217 1967
9 Ege P Transient undulations of blood glucose concentration in healthy persons Nord Med 79 645 1967
10 Exton, J H Jefferson, L S Butcher R. W & Park, C R. Gluconeogenesis in the perfused liver Amer J Med. 40 709 1966
11 Hansen, K. M Investigations on the blood sugar in man Gad, Copenha‿en 1923
12 Holmes, P A. & Mansour T E Glucose as a regulator of glycogen phosphorylase in rat diaphragm. Biochim biophys. Acta (Amst.) 156 266 1968
13 Koh C S Kohn J., Catt, K. J & Burger H G Lack of relation between plasma growth hormone levels and small decrements in blood sugar Lancet 1 13 1968
14 Kosaka, K., Mizuno Y., O‿ata, Y & Kuzuya, N Studies of glucose metabolism immediately followin‿ total pancreatectomy Diabetes 15 179 1966
15 Luft, R., Cerasi, E Madison, L L. von Euler U S., Casa, L D & Roovete A. Effect of a small decrease in blood glucose on plasma growth hormone and urinary excretion of catecholamines in man. Lancet 2 254 1966
16 Mortimore G E Effect of insulin on re ease of glucose and urea by isolated rat liver Amer J Physiol 204 699 1963
17 Robin, E D Travis, D M Julian, D G & Boshell, B R. Metabolic patterns during physiologic sleep I. Blood glucose regulation during sleep in normal and diabetic subjects. J clin. Invest. 38 2229 1959
18 Rudeman, N B & Herrera, M G Glucose regulation of hepatic gluconeogenesis. Amer J Physiol. 214 1346 1968
19 Sakata, K., Hayano S & Slovtter H A. Effect on blood glucose concentration of changes in availability of glucose to the brain. Amer J Physiol 204 1127 1963
20 Seltzer H S., Allen, E W., Herron, A. L. & Brennan, M T Insulin response in relation to glycemic stimulus. J clin. Invest. 46 323 1967
21 Sokal, J E Glucagon—an essential hormone Amer J Med. 41 331 1966
22 Sokal, J E., Sarcione E J & Henderson, A. M. Relative potency of glucagon and epinephrine as glycogenolytic a‿ents Studies with the isolated perfused rat liver Endocrinology 74 930 1964
‿3 Soskin, S., Allweiss, M D & Cohn, D J Influence of the pancreas and the liver upon the dextrose tolerance curve Amer J Physiol. 109 155 1934
24 Steele R. The influence of insulin on the hepatic metabolism and glucose Ergebn. Physiol. 57 91 1966
25 Svendsgard E. Blood sugar in normal and sick children. Levin & Munksgaard Copenhagen 1931
26 Treptow K. Hecht, K. & Baumann R. Kurzfristige Regulationsschwankungen des Blutzuckers in Nüchternkurven beim Hund. Acta biol med. germ. 15 417 1965
27 Unger R. H & Eisentraut, A M Studies of the physiologic role of glucagon Diabetes 13 563 1964
28 Vance J E Buchanan, K. D Challoner D R. & Williams, R. H Effect of glucose concentration on insulin and glucagon release from isola‿ed is ets of Langerhans of the rat. Diabetes 17 187 1968
29 Weber G Convery H J H., Lea, M A. & Stamm, N B Feedback inhibition of key glycolytic enzymes in liver action of free fatty acids Science 154 1357 1966
30 Williams R. H & Ensinck, J W Secretion, fates and actions of insulin and related products. Diabetes 15 623 1966
31 Williamson J R. Mechanism for the stimulation in vivo of hepatic gluconeogenesis by glucagon. Biochem. J 101 110 1966
32 Williamson, J R. Browning, E T Scholz, R. Kreisberg, R. A. & Fritz, I B Inhibition of fatty acid stimulation on gluconeo‿enesis by (+)-decanoylcarnitine in perfused rat liver Diabe‿es 17 194 1968
33 Wrenshall G A., Vranic M Cowan, J S & Rappaport, A. M Effects of sudden deprivation and restoration of insulin secretion on glucose metabolism in dogs. Diabetes 14 689 1965
34 Zampa, G A. Altilia F., Bracchetti D., Borgatti, E. & Odifreddi, M T Studies on peripheral glucose metabolism using the experimental forearm preparation. Diabetologia 3 35 1967
35 Zukoski, C F Mechanism of action of insulin hypoglycaemia on adrenalin cortical secretion Endocrinology 78 1264 1966

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# LACTULOSE TREATMENT OF CHRONIC HEPATOPORTAL ENCEPHALOPATHY

## *A Clinical and Electroencephalographic Study*

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*Abstract* Current treatment of chronic hepatoportal encephalopathy with dietary protein restriction is not always sufficient, and long term administration of antibiotics may lead to some undesirable side-effects The present study of three patients with encephalopathy and surgical portacaval shunt demonstrates the favourable effect of a synthetic disaccharide (lactulose Duphalac) This study also demonstrates clearly that there is a close interrelation between the EEG and the metabolism of the brain. Thus the EEG analysis can be used as a method for checking the effects of therapy in hepatic encephalopathy

Protein restriction and neomycin have for a long time had a dominating place in the treatment of chronic hepatoportal encephalopathy This therapy aims at inhibiting the bacterial degradation of proteins in the colon in order to reduce the absorption of ammonia and other nitrogenous substances considered to be responsible for the toxic symptoms In long term treatment there are however often side-effects Thus the protein restriction accentuates the negative nitrogen balance and neomycin may cause malabsorption (13) and staphylococcal enterocolitis (1) If absorbed neomycin may produce neurotoxic and nephrotoxic effects (11) Surgical treatment with colectomy has also been tried but the operation mortality is high—up to 50 % (25) The attempts to achieve a medical colectomy seem a more acceptable alternative This implies a change of the bacterial flora in the intestine in such a way that the toxic nitrogenous compounds diminish. A milk and cheese diet (6) and administration of Lactobacillus acidophilus (12 17) have been suggested as having such an effect A similar mechanism is probably responsible for the effect in those cases which have been successfully treated with the synthetic disaccharide lactulose the 1 4-$\beta$ galactosidofructose (2) This disaccharide cannot be hydrolysed by the disaccharidases in the small intestine (3) but passes unabsorbed down into the colon where it is split by bacterial action with formation of mainly lactic and acetic acids (7) The acidification of the intestine apparently reduces the absorption of ammonia and other nitrogenous compounds thought to be responsible for encephalopathy (4)

The present report describes three cases of chronic hepatoportal encephalopathy in which the neuropsychiatric symptoms were controlled by the administration of lactulose and in which changes in the clinical status were correlated to the frequency content in the EEG

## MATERIAL AND METHODS

Three cases with surgical portacaval anastomosis were selected for the study Previously they had all been treated with protein restriction, but only one had so far been treated with neomycin. During the initial period of the study they were all hospitalized but not kept in bed After leaving the hospital they continued the lactulose treatment and returned for regular controls at the out patient department. All patients were observed for several treatment periods of varying length. Laboratory analyses and EEG-examinations were carried out at the end of each period

In all the cases it was possible to start the trial with a period of only protein restriction. If the condition returned to normal an exacerbation was induced by increasing the dietary protein Not until then was lactulose given, usually in a dose of 20 ml three times a day The dose was subsequently increased to a level which produced 2-3 half-solid defaecations per day The first

observations were recorded after one week and the treatment was continued until no further improvement could be seen In two of the cases lactulose therapy was then discontinued without any other change in the regimen However because the condition deteriorated the treatment was resumed after about three weeks

Lactulose was administered in the form of an acidulous syrup (Duphalac supplied by AB Ferrosan Malmo Sweden) (pH 3 6–3 9) containing 66 7 g lactulose and 11 g galactose per 100 ml Other drug treatment chiefly thiazide diuretics was kept constant but no antibiotics were given during the time of the study

The neuropsychiatric symptoms were graded according to Parsons Smith et al (14) The EEG records were analyzed according to conventional clinical principles In addition manual frequency analysis was performed and the *EEG index* (mean period EEG frequency) was calculated from representative 20 sec samples (22) The correlation between this parameter and the clinical and laboratory findings was investigated

The EEG index is defined as the ratio between the number of all counted waves with an amplitude above 5 $\mu$V and the accumulated activity time for frequency classes from 1–20 c/sec within a 20 sec EEG sample as expressed by the formula

$$\frac{\sum_{f=1}^{2} n_f}{\sum_{f=1}^{0} n_f \dfrac{\frac{1}{cf-0.5}+\frac{1}{cf+0.5}}{2}} = \text{mean period frequency } (\bar{F}_p)$$

1– 0 = frequency classes in c/sec
$n_f$ = number of waves present in a frequency class
$cf$ = centre frequency in corresponding frequency classes

The details of the analysis technique and computations have been described elsewhere (22)

## CASE REPORTS

*Case 1* (Fig 1 *a* *b* and Fig 2)

Female aged 66 who was admitted to the hospital after a short period of diarrhoea fatigue and loss of weight A pancreatic carcinoma was suspected but laparotomy revealed no tumour The liver however had a cirrhotic appearance and biopsy showed cirrhosis of the portal type There were no postoperative complications and she improved on corticosteroids Reexamination six months later revealed however oesophageal varices For this reason the patient was operated and a portacaval anastomosis was performed The preoperative EEG was normal (frequency index 9 0) and there were no signs of cerebral disorder One year after the operation the first symptoms of encephalopathy appeared impaired memory function tiredness dizziness and episodic confusion The patient now had an abnormal EEG with a frequency index between 3 and 4 She was hospitalized for a short period and the dietary protein restriction was sufficient to improve the cerebral state and to normalize the EEG She was discharged without signs of encephalopathy

During the following year the patient kept fairly well on a low protein diet but successively developed signs of encephalopathy although she had apparently not changed her dietary regimen She complained of fatigue impaired memory and showed hyperreflexia flapping tremor and hyperkinetic dysmimia when she was readmitted During the last few months she had been unable to run her home without help

*Case 2* (Fig 3)

Male with a long history of alcohol abuse At the age of 58 a liver cirrhosis was diagnosed One year later a surgical portacaval shunt was performed because of bleeding oesophageal varices Preoperatively there were no demonstrable signs of encephalophaty either clinically or according to the EEG Two weeks after the operation he developed encephalopathy with tiredness impaired ability to concentrate and brief spells of confusion particularly during the night He was readmitted improved rapidly on a low protein diet and was discharged free of symptoms During the next two years he was hospitalized on nine occasions because of grave symptoms of encephalophaty and precoma Following the admissions the patient improved and the abnormal EEG normalized rapidly on a low protein diet and neomycin Long term treatment with neomycin was never tried He was kept on a low protein but otherwise unrestricted diet also during the periods when he was at home When the patient was readmitted for the tenth time in hepatic precoma he was considered in need of a more effective long term therapy and lactulose treatment was started

*Case 3* (Fig 4)

Female who at the age of 23 was taken ill with abdominal pain and icterus A biopsy showed changes resembling those seen in lupoid hepatitis together with early cirrhosis Corticosteroid treatment was instituted and has continued ever since The patient soon improved and could return to her previous employment Four years later the patient returned with bleeding oesophageal varices and a portacaval anastomosis was performed Preoperatively she showed no signs of encephalopathy During the following four years she was able to work and had no distressing symptoms but later developed progressive signs of encephalopathy and slowing of the EEG She could no longer manage her work because of unsteadiness and tremor in her hands In addition her ability to concentrate was reduced and her speech was slurred when she was admitted for a trial on lactulose 5 1/2 years after the portacaval shunt operation

## RESULTS

Treatment length of observation periods clinical findings and laboratory data are shown in Tables I II and III The relation between EEG frequency content (frequency polygons) and clinical course is illustrated in Figs 1 *a* 1 *b* 3 and 4 Statistical correlations between the EEG index and clinical laboratory data are given in Table IV

Table I *Clinical details in case 1*

| | Treatment periods (number of days) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | I (10) | II (14) | III (7) | IV (39) | V (186) | VI (18) | VII (9) | VIII (13) | IX (21) | X (9) | XI (177) |
| **Treatment** | | | | | | | | | | | |
| Duphalac (ml/day) | 0 | 0 | 75 | 40 | 75 | 0 | 75 | 90 | 0 | 90 | 90 |
| Dietary proteins (g day) | 50 | 80 | 80 | ? | ? | ? | ? | 70 | 70 | 70 | |
| **Encephalopathy rating** | | | | | | | | | | | |
| Clinical grade | I | II | I | I | I | II | I | I | II | I | I |
| EEG frequency index in c/sec | 7 | 4 9 | 5 6 | 6 8 | 5 7 | 5 1 | 7 2 | 7 3 | 5 0 | 5 5 | |
| **Laboratory findings** | | | | | | | | | | | |
| Serum urea (mg/100 ml) | 8 0 | 7 5 | 9 5 | 8 5 | 9 0 | | 9 0 | 11 5 | 11 0 | | 7 5 |
| Plasma sodium (mEq/l) | 135 | 138 | 139 | | | | | | | | |
| Plasma potassium (mEq/l) | 3 6 | 3 2 | 3 5 | 1 0 | 3 9 | | 3 7 | 3 ? | 3 6 | 3 ? | 3 4 |
| Serum calcium (mEq/l) | | | | | | | | 4 3 | 3 8 | 4 0 | |
| Serum phosphorus (mEq/l) | | | | | | | | 4 6 | 3 0 | 4 4 | |
| Serum magnesium (mEq/l) | | | | | | | | 1 6 | 1 5 | 1 6 | |
| Blood pH | | | | | | | | 7 43 | 7 44 | 7 52 | |
| Blood pCO (mm Hg) | | | | | | | | 35 | 32 5 | 25 5 | |
| Standard $HCO_3$ (mEq/l) | | | | | | | | 23 5 | 2 7 | 21 5 | |
| Serum bilirubin (mg/100 ml) | 2 3 | 2 ? | 2 6 | ? 1 | 2 4 | | 2 4 | 2 3 | 2 0 | 5 | 2 4 |
| Serum GOT (units) | 73 | 130 | 98 | | 76 | | 58 | 62 | 73 | 100 | |
| Serum GPT (units) | 34 | 100 | 60 | | 46 | | 35 | 30 | 39 | 40 | |
| Serum LDH (units) | | | | | | | | 4 0 | 500 | 340 | |
| Alkaline phosphatase (units) | | | | | | | | 41 | 10 | 8 | |

Fig 2 demonstrates the impairment of motor performance (drawing of a 5 pointed star) by case 1

In all three patients the lactulose treatment was preceded by a period of dietary protein restriction During this period all patients improved clinically and their EEG abnormalities diminished In order to obtain more convincing results dietary restrictions were relieved and the daily protein

Table II *Clinical details in case 2*

| | Treatment periods (number of days) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | I (7) | II (7) | III (7) | IV (7) | V (7) | VI (14) | VII (14) | VIII (14) | IX (28) | X (40) |
| **Treatment** | | | | | | | | | | |
| Duphalac (ml/day) | 0 | 0 | 120 | 1 0 | 0 | 0 | 90 | 150 | 150 | 150? |
| Dietary proteins (g day) | 0 | 80 | 80 | 80 | 80 | 80 | 0 | 50 | 60 | 60 |
| **Encephalopathy rating** | | | | | | | | | | |
| Clinical grade | I | II | I | I | I | I | III | I | II | II |
| EEG frequency index in c sec | 10 7 | 5 8 | 10 1 | 11 5 | 6 5 | 5 0 | 4 5 | 6 6 | 5 7 | 5 ? |
| **Laboratory findings** | | | | | | | | | | |
| Serum urea (mg/100 ml) | 8 0 | 11 5 | 10 0 | | 8 0 | | 6 0 | 12 5 | | 9 0 |
| Plasma sodium (mEq/l) | 135 | 137 | | 136 | 152 | 143 | 141 | 143 | 141 | 134 |
| Plasma potassium (mEq/l) | 3 5 | 3 5 | | 3 6 | 3 4 | 3 4 | 3 2 | 3 1 | 3 9 | 3 0 |
| Serum calcium (mEq/l) | | | | 4 3 | 3 9 | 4 5 | 4 3 | 4 0 | | |
| Serum phosphorus (mEq/l) | | | | 2 3 | 2 6 | 3 2 | 3 4 | ? 5 | | |
| Serum magnesium (mEq/l) | 1 7 | 1 9 | | 1 7 | 1 5 | | 1 8 | 1 8 | | |
| Blood pH | 7 43 | 7 47 | 7 45 | 7 46 | 7 49 | 7 38 | 7 47 | 7 49 | 7 48 | 7 55 |
| Blood pCO (mm Hg) | 34 0 | 8 0 | 32 5 | 25 5 | 24 0 | 30 5 | 25 0 | 9 0 | 6 5 | 26 5 |
| Standard HCO (mEq/l) | 3 4 | 22 7 | 24 0 | 25 2 | 22 1 | 19 6 | 19 0 | 21 0 | 0 1 | 24 6 |
| Serum bilirubin (mg/100 ml) | 1 4 | 2 0 | | 1 2 | 1 1 | 1 9 | 2 2 | 2 2 | 1 7 | 2 0 |
| Serum GOT (units) | 35 | 31 | 45 | 35 | 41 | | 3. | 40 | 45 | 62 |
| Serum GPT (units) | 28 | 27 | 25 | 21 | 19 | | 36 | ?3 | 24 | 35 |
| Serum LDH (units) | | | | | 360 | 350 | 480 | 4 0 | | |
| Alkaline phosphatase (units) | 3 | 6 | | 6 | 6 | 8 | 8 | 6 | | |

Table III *Clinical details in case 3*

| | Treatment periods (number of days) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | I (7) | II (8) | III (8) | IV (22) | V (17) | VI (19) | VII (28) | VIII (28) | IX (70) | X (63) |
| Treatment | | | | | | | | | | |
| Duphalac (ml/day) | 0 | 0 | 60 | 75 | 90? | 90? | 5? | 75? | 60 | 60 |
| Dietary proteins (g/day) | 60 | 80 | 80 | 80 | 60? | 60? | 60? | 60? | 60 | 60 |
| Encephalopathy rating | | | | | | | | | | |
| Clinical grade | I | II | I | 0 | I | 0 | I | 0 | 0 | 0 |
| EEG frequency index in c/sec | 9 7 | 5 6 | 8 8 | 11 7 | | | | | | |
| Laboratory findings | | | | | | | | | | |
| Serum urea (mg/100 ml) | 13 5 | 9 0 | 9 5 | 13 0 | 13 5 | 13 0 | 14 0 | 18 5 | 12 5 | |
| Plasma sodium (mEq/l) | 128 | 134 | 140 | 138 | | | | | | |
| Plasma potassium (mEq/l) | 3 5 | 3 3 | 3 3 | 3 2 | | 2 9 | 5 0 | 2 6 | 2 8 | |
| Serum magnesium (mEq/l) | | | | 1 4 | 1 5 | | | | | |
| Blood pH | 7 41 | 7 42 | 7 40 | 7 41 | | | | | | |
| Blood $pCO_2$ (mm Hg) | 30 5 | 30 0 | 31 0 | 40 | | | | | | |
| Standard $HCO_3$ (mEq/l) | 20 8 | 20 5 | 20 0 | 24 3 | | | | | | |
| Serum bilirubin (mg/100 ml) | 5 6 | 2 8 | 3 4 | 3 4 | 3 2 | | 2 6 | 4 4 | 3 5 | 3 6 |
| Serum GOT (units) | 275 | 145 | 141 | 88 | 49 | | | | | |
| Serum GPT (units) | 195 | 119 | 104 | 73 | 24 | | | | | |
| Alkaline phosphatase (units) | 8 | | 5 | | | | | | | |

intake increased by 20–30 g before the trial with lactulose was started This resulted in a clear cut exacerbation of the neuropsychiatric symptoms and in a slowing of the EEG While still maintaining the protein intake on this higher level the lactulose treatment was started This produced a regress of the clinical symptoms to about the same stage as before the dietary protein was increased Also the EEG improved with return of the mean frequency to about the same level as before increasing the protein content in the diet In two of the cases these values were even exceeded When lactulose was withdrawn in two of the cases without any change in the daily diet more pronounced symptoms of encephalopathy reappeared clinically as well as in the EEG

When the initial trial had been completed the patients were discharged with dietary instructions implying moderate protein restriction and told to continue with lactulose according to the dosage

Table IV *Product moment correlation between EEG frequency content and some chemical laboratory tests*

| Laboratory test | Covariable (mean value) | EEG frequency index (mean value) | *n* | *r* | *p* |
|---|---|---|---|---|---|
| Standard bicarbonate | 22 1 | 7 4 | 17 | 0 53 | 3 |
| Arterial $pCO_2$ | 30 4 | 7 4 | 17 | 0 51 | 4 |
| Magnesium in serum | 1 8 | 7 6 | 9 | 0 60 | n s |
| Urea in serum | 9 7 | 7 1 | 19 | 0 39 | n s |
| Blood pH | 7 6 | 7 4 | 17 | 0 37 | n s |
| Phosphorus in serum | 4 2 | 6 5 | 8 | 0 35 | n s |
| Sodium in plasma | 138 0 | 7 2 | 16 | 0 32 | n s |
| Bilirubin in serum | 2 4 | 6 9 | 22 | 0 22 | n s |
| Calcium in serum | 4 2 | 6 5 | 8 | 0 18 | n s |
| SGPT | 44 6 | 7 2 | 21 | 0 14 | n s |
| SGOT | 80 2 | 7 2 | 21 | 0 05 | n s |
| Potassium in plasma | 3 4 | 6 9 | 22 | 0 01 | n s |

n s = statistically not significant

*Fig 1a* Case 1 The upper diagram shows the variations of the mean period EEG frequency (indicated by filled circles) in relation to therapy *during the initial period* of lactulose treatment The daily protein intake was controlled only during the first part of the period (Question marks above dotted line indicate non-controlled protein intake) The lower diagrams illustrate the spectral distribution of the EEG activity in the stages indicated in the upper diagram. Only the EEGs with the lowest and highest mean period frequency in the same series are illustrated. $T_4$–O indicates that the electrodes were placed in the occipitotemporal region according to the 10–20 Electrode System of the International EEG Federation (9)

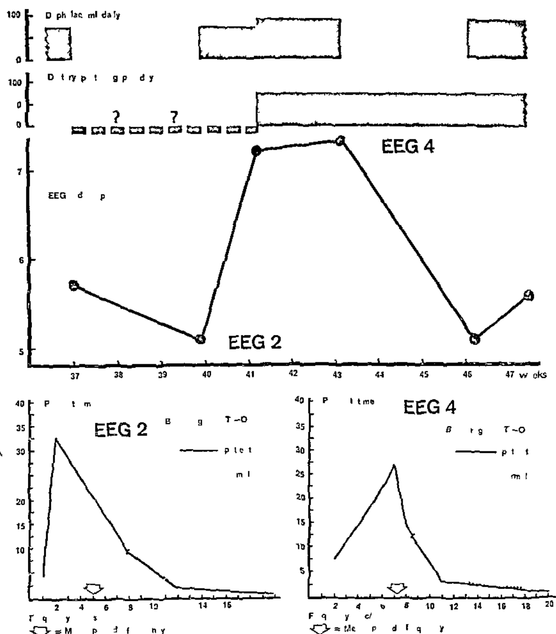



*Fig 1 b* Case 1 The upper diagram shows the variations of the mean period EEG frequency in relation to intermittent therapy *during prolonged treatment* The lower diagrams show the spectral distribution of the EEG activity in the same manner as in Fig 1 *a* For explanation see Fig 1 *a*

that had been found to produce the best result Because no specified dietary regimen was given it was not possible to calculate the exact protein consumption between admissions

*Case 1* was able on the lactulose to resume her daily activities at home after leaving the hospital However she did not follow the medical instructions consistently and for some time the lactulose dose was lower than prescribed Yet there was no marked deterioration in her mental condition until she decided to stop taking lactulose completely After that there was a rapid development of progressive encephalopathy reflected clearly also in the EEG frequency pattern Lactulose was reinstituted after which her condition improved She was later readmitted to the hospital for a new attempt to perform the treatment under controlled conditions When lactulose was with

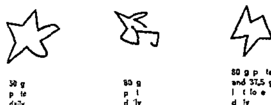
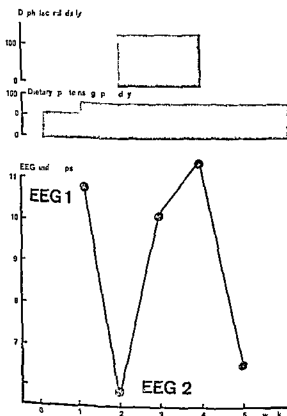



*Fig* ? Case 1 Attempt to draw a 5 pointed star before and during lactulose therapy

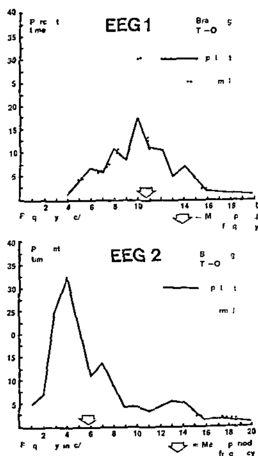
drawn without any other change in her regim n a new deterioration ensued (Fig 1 *b*) Aft^r resum ing the lactulose treatment sh again improved She has subsequently been taking lactulose regu larly in a dose which has been increased succes sively up to 90 ml daily In addition she has kept a low protein diet but otherwise she has not been under any d tai'ed dietary regimen She has now been treated for more than two years and her improvement and working capacity have been maintained with the exception of the episode described above when no lactulose was taken Also in this patient the variations in the cerebral state were adequately reflected in the EEG




*F* 3 Case The left d a ram shows the var ations in the m an period EEG frequency in relation to therapy

*Case 2* made good progress at first after leaving hospital However already after about two weeks th re was a deterioration and slowing of the EEG despite continued therapy Nevertheless the symptoms regressed and the EEG became normalized after increased lactulose dosage and reduced pro tein intake He spent some time in a convalescent home and kept fairly well until an exacerbation two months later brought him back to the hospital in a state of p ecoma Despite continued treatment with lactulose with added neomycin and protein free diet his deterioration progressed the EEG b came very abnormal and he finally died Au topsy showed p onoun ed atrophic liver cirrhosis of the portal type

*Case 3* has stayed well and her EEG stabilized with the spectral d str but on illu trated in the diagrams to the right as in Fig. 1 *a* and 1 *b*



## REFERENCES

1 Alder P Cited by Read A E McCarty C F Heaton K. W & Laidlow J Brit med J 1 1267 1966
2 Bircher J Miller J Guggenheim, P & Haemmerli U P Lancet 1 890 1966
3 Dahlqvist A & Gryboski J O Biochim biophys Acta 110 635 1965
4 Ellington S G Floch M H & Conn H O Lactulose in the treatment of chronic portal systemic encephalopathy To be published
5 Fazekas J F Ticktin H E Ehrmantraut, W R. & Alman A Amer J Med 21 843 1956
6 Fenton J C B Knight, E J & Humpherson P L. Lancet 1 164 1966
7 Hoffman K Mossel D A A Korus W & van de Kamer J H Klin Wschr 42 126 1964
8 Ingvar D H & Sulg I A Scand J Clin lab invest Suppl 109 49 1969
9 Jasper H H Electroenceph clin Neurophysiol 10 371 1958
10 Kuhn A Dtsch Med Wschr 83 658 1958
11 Last P M & Sherlock S New Engl J Med 262 385 1960
12 Macbeth W A A G Kass E H & McDermont W V Lancet 1 399 1965
13 Pass I C Searl P Rubert M W & Falcon W W Gastroenterology 53 49 1967
14 Parsons Smith B G Summerskill W H J Dawson A M & Sherlock S Lancet 2 867 1957
15 Perrin H Fortschr Neurol Psychiat 35 174 1967
Posner J B & Plum, F J clin Invest 39 1246 1960
Read A E McCarty C F Heaton K W & Laidlow J Brit med J 1 1267 1966
18 Riddell A G Ann roy Coll Surg Engl 17 1605 1955
19 Sherlock S Summerskill W H J White L P & Phear E A Lancet 2 453 1954
20 Sherlock S Amer J Med 24 805 1958
21 — Ann Rev Med 11 47 1960
22 Sulg I A Scand J Clin lab Invest Suppl 109 1969
23 Sulg I A & Ingvar D H Electroenceph clin Neurophysiol 19 615 1965
24 Waelsch H Berl S Clarke D D Tagaki G & Purpura D P Proceedings Seventh Internationl Congress of Neurology Rome 1961 Neurochemistry Symposium pp 165–169 Amsterdam 1963
25 Walker J G Embyn Williams A Craigie A Rosenoer W M Agnew J & Sherlock S Lancet 2 861 1965
26 Walshe J M Quart J Med 20 421 1951

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# METABOLIC STUDIES OF FOLIC ACID IN NON MALIGNANT DISEASES[1]

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*Abstract* The effect upon the serum folic acid activity (SFAA) of acute illness and chronic illness has been studied in 173 persons. Old, healthy subjects getting proper meal from institutional kitchen have normal SFAA, while those preparing their own food have low SFAA. The SFAA is normal in acute illness (thyreotoxicosis, fractures, after operations) In chronic inflammatory diseases (pulmonary tuberculosis, rheumatoid arthritis) low or subnormal SFAA is common. In rheumatoid arthritis patients the SFAA is lower the more active the disease and the higher the erythrocyte sedimentation rate Serum iron and serum cholesterol are also low and correlated to both SFAA and sedimentation rate The intestinal absorption of folic acid is normal in patients with rheumatoid arthritis, while the plasma clearance of folic acid is greatly increased. The high frequency of subnormal SFAA in this group may be explained by inadequate diet and or increased turnover of folic acid in the plasma.

Our knowledge of the alterations in the nutrient metabolism in various diseases is scanty Theories of an *increase in the consumption of vitamins*—for instance in infectious diseases—have been exploited more to promote the sale of vitamin tablets than as a basis for extensive research The erythropoietically active vitamins are among those whose metabolism is best understood and modifications of Hunneken s method for determination of folic acid in serum have provided a useful instrument in research

Apart from the expected folic acid deficiency in sprue and pregnancy an unexpectedly low serum folic acid activity (SFAA) has been reported in cases of carcinoma and other malignant neoplasms (3 10 17 18) and in haemolytic anaemia In addition low SFAA or other signs of folic acid deficiency occur in some chronic inflammatory conditions such as rheumatoid arthritis (2 4–6 16) and possibly in certain liver diseases

The aim of this study was to examine whether persons who are ill or elderly generally have a low SFAA as a non specific sign of disease or whether low SFAA is specific for certain groups of diseases In addition a study was made of the relation between SFAA and other clinical parameters in rheumatoid arthritis in order to find a possible correlation between the SFAA and the activity of the rheumatic disease Low SFAA may be due to a reduced intake of folic acid to an impaired intestinal absorption of folic acid or to an acceleration of the plasma clearance For this reason the intestinal absorption and the plasma clearance of folic acid was also studied in patients with rheumatoid arthritis A study of the effects of different diets has not yet been performed

## MATERIAL

The study was carried out on nine groups [illegible] al [illegible] (Table I) Groups 1 [illegible] consisted of healthy subjects of different ages [illegible] group 1 there were [illegible] group 2 consisted [illegible] 4 retired persons at the Dan ken Old People Home and [illegible]0 patients from the Departments of S[illegible] and O[illegible] at the Karolinska Hospital These [illegible]0 patients suffered from minor [illegible] diseases [illegible] which did not affect their general health but made it difficult for them to live at home without practical difficulties [illegible] during a short period of treatment at the hospital In group 3 there were another ten retired persons from the Naesta Pensioner Apartments The subjects in group 3 prepared their own food, while the others in group 1 [illegible] received practically all *all their meals from institutional* kitchen

Groups 4–7 consisted of patients with various diseases In group 4 there were 13 cases of coronary thrombosis, all with typical ECG and transaminase reactions The

[1] Preliminary reports have been published at the Annual Congress of the Swedish Physicians Association in 1963 (4) and 1964 (5)

Table I *Clinical material*

| Subjects | No of subjects | Age Mean | Age Range | Mean SFAA (mμg/ml serum) | S D | S E | No of subjects with SFAA below 2 3 (mμg/ml serum) |
|---|---|---|---|---|---|---|---|
| 1 Healthy young subjects | 16 | 25 | 22–35 | 5 0 | 1 32 | 0 38 | 0 |
| 2 Healthy elderly with institutional kitchen | 44 | 76 | 60–88 | 5 2 | 1 78 | 0 27 | 0 |
| 3 Healthy elderly who prepare their own food | 10 | 75 | 71– 8 | 2 8 | 0 96 | 0 30 | 3 |
| 4 Myocardial infarction | 13 | 64 | 44–78 | 3 4 | 1 32 | 0 44 | 1 |
| 5 After gallbladder operations | 9 | 42 | 25–68 | 7 0 | 3 19 | 1 06 | 0 |
| 6 Fractures | 9 | 72 | 59–80 | 5 1 | 1 72 | 0 57 | 0 |
| 7 (*a*) Thyreotoxicosis | 13 | 55 | 41–68 | 4 , | 2 37 | 0 56 | 1 |
| (*b*) Severe thyreotoxicosis | 5 | 49 | 43–55 | 4 9 | 3 50 | 1 57 | 0 |
| 8 Chronic active pulmonary tuberculosis | 8 | 31 | 23–39 | 3 1 | 0 65 | 0 32 | 0 |
| 9 Rheumatoid arthritis | 46 | 50 | 15–78 | 3 76 | 2 87 | 0 43 | 18 |

SFAA was determined within the first week after the onset of their illness In group 5 there were nine patients who had undergone gall-bladder operations due to cholelithiasis. The SFAA was determined within 24 hours after the operation. In group 6 there were nine patients who had undergone surgical treatment due to fractures. Most of them had had fractures of the femoral neck treated with fixation with nails. The SFAA was determined within one week after the operation In group 7 there were 18 patients with thyreotoxicosis five of whom selected cases with threatening toxic crises In ...ps 8–9 there were eight cases of chronic active tuber~ulosis and 46 cases of rheumatoid ... The diagnosis rheumatoid arthritis was made ...g to the criteria of A.R.A. (,0 21) In 36 of these 46 patients the correlations between the SFAA and other laboratory parameters were mapped 15 of these 36 had subnormal SFAA so that the relative distribution between patients with normal and subnormal SFAA remained unchanged compared to that in the whole group of 46 patients

At the time of the examination the patients had not received any treatment that as far as was known, could affect the SFAA analysis (19) Thus no chemotherapy antibiotic or antiepileptic therapy had been given. The patients with tuberculosis received para amino-salicylic acid and isoniazide In vitro tests showed however that these substances have no effect on the growth of Lactobacillus casei. Reticulocytes and serum bilirubin were normal in all cases, so appreciable haemolysis in the examined subjects can probably be ruled out. Patients with complicating diseases were excluded

## METHODS

The SFAA analyses were performed by the microbiological method with Lactobacillus casei described earlier (19) Bacterial growth in a described folic acid free medium

Table II *Plasma clearance of folic acid in healthy subjects and patients*

| References | Amount of folic acid injected | No of persons | Serum concentration (mμg/ml) after 3 min | 15 min | 30 min | Test organism |
|---|---|---|---|---|---|---|
| Hansen and Nystroem 1964 (8)[a] | 15 μg/kg body weight | ? | 70–,00 | 20–75 | 5–55 | S fecalis[b] |
| Grossowicz et al 1962 (7) | 1 mg | 7 | 106 (61–200) | 53 (61–112) | 27 (12–56) | S fecalis |
| Chanarin and Bennett 1965 (1) | 15 μg/kg | 50 | 151±6 | 45±3 | 21±2 | S fecalis |
| Johns et al 1961 (11) | 15 μg/kg[d] | ? | 6 ,. of the dose/l | 3 | 1 8 | H PCA |
| Metz et al 1961 (12) | 15 μg/kg | 21 | 152±44 | 48±17 | 22±3 6 | S fecalis |
| Metz et al 1961 (12) | 15 μg/kg | 13 | 151±22 | 51±11 | 27±9 6 | S fecalis |
| Herbert and Zalusky 1962 (9) | 15 μg/kg | 3 | >100–>300 | >92–100 | 57–60 | L. casei |
| Einhorn and Reizenstein 1966 (3) | 15 μg/kg | 10 | 3,0±24 | 136±5 | 76±9[c] | L casei |
| Present study | 15 μg/kg | 33 | 201±12 | 72±6 | 44±3[c] | L casei |

[a] No difference between men and women
[b] No difference between S fecalis and L casei
[c] Standard error
[d] When 1 μg/kg given clearance was slower clearance faster when 150 μg/kg given

was measured in duplo in three dilutions of each sample and the corresponding folic acid content was calculated on the basis of standard curves obtained for each analysis. The serum samples were taken from the fasting patient with acid washed steel cannulas into specially folic acid free tubes containing two mg of ascorbic acid. The initially high concentration of ascorbic acid was found not to affect the folic acid concentration. The analyses were performed within four hours of the specimen being taken. A few serum samples, however were kept for a few days after immediate freezing to −20 C.

The in vitro effect on Lactobacillus casei growth of pharmacological concentrations of para amino-salicylic acid (0 01 mg/ml) and isoniazide (0 001 mg/ml) was examined as described (19).

The intestinal absorption of folic acid was examined in six healthy subjects aged 23–31 (mean 25) and in six patients with rheumatoid arthritis and subnormal SFAA, aged 51–79 (mean 61). After an initial venous sample the subjects were given folic acid perorally about 40 μg/kg of body weight. New venous samples were taken after one and two hours. The subjects were kept fasting during the entire test.

The plasma clearance of folic acid was examined in 33 healthy young subjects and in 13 patients with rheumatoid arthritis aged 23–66 (mean 52) six of whom had subnormal SFAA, aged 52–64 (mean 56). After initial venous samples folic acid was given intravenously about 15 μg/kg of body weight. Further venous samples were taken 3 15 and 30 minutes later from different veins in the other arm. The subjects were kept fasting during the entire test.

The normal values for plasma clearance of folic acid were found to vary appreciably from one laboratory to the other (Table II) but also within the same laboratory

Table III *Technical variations in folic acid plasma clearance values in healthy controls (means ± S E of mean)*

| Effects studied | No of cases | SFAA (mμg/ml) at various times after folic acid injection: 3 min | 15 min | 30 min |
|---|---|---|---|---|
| Time variations | | | | |
| 1960[a] | 10 | 320±24 | 136±15 | 76±9 |
| 1962 | 10 | 225±28 | 89±15 | 54±9 |
| 1965 | 8 | 196±23 | 67±5 | 36±5 |
| 1966 | 13 | 186±13 | 63±4 | 38±3 |
| Sex | | | | |
| Males | 16 | 187±20 | 65±3 | 40±2 |
| Females | 6 | 184±18 | 61±9 | 36±5 |

One technician performed the assays prior to 1962, another after 1962. New strains of Lactobacillus casei ATCC 7469 were purchased at intervals

(Table III). Although such variations are not uncommon where bioassays are concerned an attempt was made to elucidate their cause. Differences between different microorganisms (Table II) and techniques (Table III) seemed to account for most of the variations, but variations from one year to the other also occurred (Table III). Sex differences could not be demonstrated (Table III).

*Statistical analysis*

The linear correlation coefficients (*r*) regression coefficients (*b*) and their standard deviations (S D ) were cal

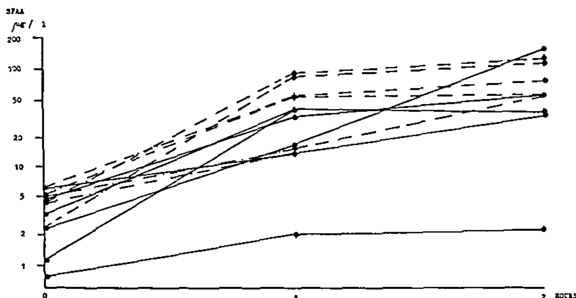



*Fig 1* Serum folic acid activity after administration of folic acid perorally in patients with rheumatoid arthritis. —— patients – – – healthy controls.

Table IV *SFAA in elderly subjects*

| Reference | No of subjects | Hospitalized = H living at old age institutions = L or at home | Mean age y (mean ± S D ) | SFAA (mμg/ml) (mean ± S D ) |
|---|---|---|---|---|
| Hansen and Nystroem 1964 (13) | 131 | H | 78 ± 6 | Low 2 8 ± 1 5[a] |
| Nystroem and Hansen 1964 and 1965 (14 15) | 157 | 136 at home | 75 ± 4 | Normal[b] |
| Present study | 44 | L | 76 ± 5 | 5 2 ± 1 78 |
| | 18 | At home | 76 ± 2 | 2 6 ± 0 98 |

[a] Whole blood folic acid 41 ± 18 6 mμg/ml as compared to normal 58 ± 20

[b] Whole blood folic acid 57 ± 20 mμg/ml in those with normal and 50 ± 20 in those with increased sedimentation rates

culated with an IBM 044 programme Coreg 2 described elsewhere (2?)

## RESULTS

Normal values for the SFAA are 2 3–7 7 mμg/ml serum (19) The normal values for plasma clearance obtained with the present subjects and patients are given in Table II The normal values for the intestinal absorption are given in Fig 1

The SFAA in elderly persons is given in Tables I and IV Subjects eating food prepared in an institutional kitchen seem to have higher SFAA than elderly doing their own cooking

There is no significant decrease in the SFAA in the acutely ill persons with the exception of the myocardial infarction group (Table I) The fracture group however displayed normal SFAA while the patients undergoing gall bladder operations had relatively high means This was due to four patients with high SFAA up to 11 mμg/ml all of whom showed signs of a rapid transient liver damage with increased bilirubin and transaminases four days after the operation

The thyreotoxic patients were normal even those with threatening crises

The patients with tuberculosis had a significantly lower mean SFAA than the healthy subjects but none of them had a subnormal SFAA The mean SFAA in the group with rheumatoid arthritis was also significantly lowered here 18 out of 46 subnormal SFAA In the rheumatic group there was also a statistically significant correlation between low SFAA and erythrocyte sedimentation rate serum iron and serum cholesterol (Table V)

Table V *Significant correlations between different parameters in patients with rheumatoid arthritis*

| | SFAA | Duration of disease | Age | Erythrocyte sedimentation rate | Serum cholesterol | Body weight | Serum iron | Rheumatoid haem agglutination test |
|---|---|---|---|---|---|---|---|---|
| SFAA | | | | | | | | |
| Duration of disease | | | | | | | | |
| Age | | 2 | | | | | | |
| Erythrocyte sedimentation rate | 2 | | | | | | | |
| Serum cholesterol | 1 | 1 | | | | | | |
| Body weight | | | | | | | | |
| Serum iron | 2 | | | 3 | | 1 | | |
| Rheumatoid haem agglutination test | | | | | | | | |
| Haemoglobin | | | | 2 | | | 1 | |

The figures indicate the degree of statistical significance of the correlation coefficient between the variable to the extreme right of the table and that at the top of the column The serum folic acid concentration is lower the higher the erythrocyte sedimentation rate the lower the serum cholesterol and the lower the serum iron are

1 = $p$ between 0 05 and 0 01

2 = $p$ between 0 01 and 0 001

3 = $p < 0\ 001$

Table VI *Plasma clearance of folic acid in patients with rheumatoid arthritis and in healthy controls*

| | Serum folic acid concentrations (mμg ml) (mean ± S E of mean) | | | Slope ( folic acid removed from plasma per min) 3–30 min |
|---|---|---|---|---|
| | 3 min | 15 min | 30 min | |
| Healthy controls | 201 ± 12 | 72 ± 6 | 44 ± 3 | 5 97 ± 0 45 |
| Rheumatic patients | 80 ± 7 | 22 ± 2 | 12 ± 1 | 7 95 ± 0 88 |
| Difference between means | 121 | 50 | 32 | 1 98 |
| Significance | 0 01 > $p$ > 0 001 | $p$ < 0 001 | $p$ < 0 001 | $p$ > 0 05 |

The absorption study (Fig 1) did not demonstrate any significant difference between the healthy group and the group with rheumatoid arthritis patients In one patient the rise in SFAA on supply of folic acid was less than in the others but she had a rapid plasma clearance which probably accounts for the slow rise more than does reduced intestinal absorption

The plasma clearance of folic acid in patients with rheumatoid arthritis was greatly increased apparently mainly during the first three minutes after the injection After that time the rheumatic patients had significantly lower means though the clearance rate was only moderately higher in the rheumatic patients

## DISCUSSION

It is difficult to account for the divergent results (Table V) as regards the SFAA in elderly subjects —differences not only between laboratories (8 13–15) but also between groups of subjects studied simultaneously in the same laboratory (Table IV) The most probable explanation nonetheless lies in differences in meal habits and food preparation One surprising feature is that subjects living at home whose food does not take the long preparation suspected in the institutional kitchen did not record higher SFAA than the subjects living at the institutions Rather it seems to be necessary for the elderly to have meals prepared for them in order to maintain a normal folate level

Acutely ill patients as a rule had normal SFAA This was true even in the group of recently operated patients who often display disturbances in the metabolism of other nutrients such as iron However a lower mean SFAA was recorded in the patients with myocardial infarction

The explanation of the large reduction in the SFAA found in patients with chronic inflammatory diseases is obscure An increased basal metabolism alone does not appear to reduce the SFAA as demonstrated by the normal SFAA in thyreotoxicosis The activity of the disease as reflected by the erythrocyte sedimentation rate seems to cause an increased turnover or tissue take up of folic acid It is interesting that iron and cholesterol are affected similarly Defective intestinal absorption does not seem to be an important factor but decreased intake may explain the low serum concentrations of folic acid It is uncertain however whether the rapid clearance and the low iron and cholesterol values can be explained in this way

## ACKNOWLEDGEMENTS

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## REFERENCES


1 Chanarin I & Bennett M C Brit J Haemat 8 95 1965

Deller D J Urban E Ibbotson R N Horwood, J Milazzo S & Robson H N Brit med J 1 65 1966

3 Einhorn J & Reizenstein P Cancer Res 26 340 1966

4 Elman A Johansson M Olhagen B & Reizenstein P Annual Congress of The Swedish Physicians Association Stockholm 1963 p 173

5 Elman A Reizenstein, P Hanngren A & Einhorn J Annual Congress of The Swedish Physicians Association Stockholm 1964 p 210

6 Gough K R McCarthy C Read A E Mollin D L & Waters, A H Brit med J 1 12 1964

7 Grossowicz, N Abronovich J Rachmilewitz, M Izak, G Sadovsky A & Bercovici B J Lab clin Med 60 375 1962

8 Hansen, H. A. & Nystroem, B Svensk Lak Tidn. 61 578 1964

Tab'

& Zalusky R. J clin Invest. 41 1263

en, B Baker H & Reizenstein P Blood 1961

11 D G Sperti, S & Burgen, A. S V J clin Invest 40 1684 1961

12. Metz, J Stevens K. Krawitz, S & Brandt V J clin. Path. 14 622 1961

13 Nystroem B Folic acid in the aged The Astra Drug Co., Stockholm 1964

14 Nystroem B & Hansen H A. Abstracts Xth Congress of the International Society of Haematology p C 27 Stockholm 1964

15 Nystroem B & Hansen H. A. Annual Congress of The Swedish Physicians Association Stockholm 1965 p 231

16 Partridge R. E H. & Duthie J J R. Brit. med J 1 89 1963

17 Rama Rao P B Lagerlof B., Einhorn, J & Reizenstein P Lancet 1 1192, 1963

18 Rama Rao P B., Lagerlof B Einhorn J & Reizenstein P Cancer Res 25 221 1965

19 Reizenstein P Acta med scand. 178 133 1965

20 Ropes, M W Bennett, G A. Cobb S Jacox R. F & Jessar R. A. Bull. rheum. Dis 7 121 1958

21 Ropes, M W Bennett, G A., Cobb S Jacox R. F & Jessar R. A. Bull rheum. Dis 9 175 1958

22. Zackrisson, L & Reizenstein P Mapping of correlation between clinical variables. In Computer applications in medical research and clinical physiology (ed. C Cederlund, A. Holmgren, P Reizenstein & T Ringqvist) IBM Stockholm 1968

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# PLASMA LIPIDS AND LIPOPROTEINS IN PATIENTS WITH MYOCARDIAL INFARCTION AND IN A CONTROL MATERIAL

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*Abst act* One hundred and eight patients with myocardial infarction have been investigated 1–3 ½ year after the acute attack. As a control group 44 coaeval hospital patients with no known ischaemic heart disease and 65 healthy young subjects were examined.

Significantly higher values were found for plasma total lipid, tr glyceride cholesterol, and phospholipids in the coronary patient group as compared with the coaeval hos pital control group and in the last mentioned group as compared with the young controls

Paper electrophoretic lipoprotein estimations were car ried out in all cases 15 9 of the hospital control group and 42 6 of the coronary patient group had Type II hyperlipoproteinaemia Hyperlipoproteinaemia with in creased pre β fraction was found in 34 1 and 35 2 respectively of the same groups. Analyses of the occurrence of pre β lipoprotein fractions in all three groups seem to suggest the ex stence of two different populations accord ing to the metabolism of endogenous triglycer des We think that this fact must be taken into consideration in future dietetic trials w th the aim of prevent ng ischaemic heart d sease

The relation b tween coronary atherosclerosis and hyperlipidaemia has been investigated by several authors Particular interest has been shown in the relationship to hypercholesterolaemia (4 16) but during recent years hypertriglyceridaemia has been found to be closely correlated with ischaemic heart disease (1 7 12 19 21 28)

Triglycerides appear in the blood mostly in two different forms in chylomicrons the metabolism of which is dependent on normal post heparin lipolytic activity (PHLA) and as very low density lipoproteins (VLDL) the metabolism of which seems to be dependent on a normal carbohydrate metabolism A defect in this integrated metab olism resulting in so-called carbohydrate induced hypertriglyceridaemia or endogenous hypertriglyc eridaemia is according to recent investigations of relatively small series found to be common in persons with ischaemic heart disease (26 28)

The relative importance of the role of faulty lipid or carbohydrate metabolism leading to hyper cholesterolaemia or hypertriglyceridaemia might be elucidated by prolonged and vast prospective examinations of people living on a relevant dietary regimen This ought to be carried out but the task is formidable and very time-consuming However it seems as if the much simpler electrophoretic analysis of the plasma lipoproteins gives some information of a possible correlation between the various forms of hyperlipidaemia and the clinical entities and metabolic disorders (14)

In patients with coronary disease estimations of the plasma lipoproteins have so far not been carried out to the same extent as other lipid analyses especially that of plasma cholesterol but in the few series published clearly abnormal lipoprotein patterns have been found not only regarding the cholesterol rich beta lipoproteins but also the triglyceride rich pre beta lipoproteins which correspond to VLDL (3 6 9 11 24) A sometimes disregarded fact is that plasma lipids during the time just after an acute myocardial infarction undergo great changes and a return to pre infarct values does not occur until several months later (5 32)

The conclusion from the observations of the above authors seems to point to carbohydrate induced hypertriglyceridaemia as a rather common metabolic disorder among persons with coronary disease whereas hypercholesterolaemia in the relation of hyperlipidaemia to ischaemic heart disease plays a less important role as emphasized earlier certain investigators even fail to find any increased mortality from ischaemic heart disease among patients with familial hypercholesterolaemia (18)

These facts combined with the great racial and

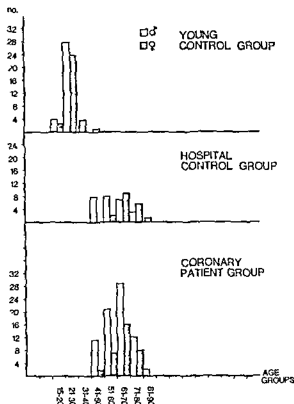



*Fig 1* Age and sex distribution in the three investigated groups

geographical differences in the levels of plasma lipids seem to warrant an investigation in a random selection of patients with ischaemic heart disease concerning the lipoprotein pattern as well as the plasma lipid levels This has been the aim of the present work

## MATERIAL

Patients admitted to the Aalborg City Hospital for myocardial infarction in the period January 1 1965 to July 31 1967 were examined—i e at least one year before the time of investigation The diagnosis of myocardial infarction was based on the following criteria typical history with chest pains, transitory ECG alterations and elevation of serum glutamic oxaloacetic transaminase and lactate dehydrogenase At least two of these criteria should be fulfilled for the diagnosis to be made

Patients with diseases known to interfere substantially with lipid metabolism, i.e hepatic diseases, hyper and hypothyreoidism, nephrosis, and treatment-dependent diabetes mellitus were excluded.

Three hundred and fifty-seven patients suffering from myocardial infarction were admitted to the hospital in the above mentioned period. One hundred and seventy four patients died before the time of investigation, and 75 patients were excluded for reasons stated above leaving 108 patients 77 males and 31 females ("coronary patient group )

As control materials were examined 44 randomly selected persons with a similar age distribution to that of the patient group hospitalized for other reasons than ischaemic heart disease and without any disease considered to interfere with the lipid and/or carbohydrate metabolism ( hospital control group") and another group of 65 young healthy persons ( young control group )

The age and sex distributions of the three groups are shown in Fig 1

## METHODS

All persons were examined in the morning after 12 hours of complete fasting The following examinations were carried out measurement of height weight, blood pressure blood glucose concentration serum protein and protein electrophoresis *Investigations of the plasma lipids* were carried out as follows immediately after the vein puncture plasma from a K EDTA stabilized blood sample was separated and lipoprotein-electrophoresis was carried out by the method of Lees and Hatch (22) and the lipoproteins were stained by the method of Jencks and Durrum (20) The total lipids of the plasma were extracted and determined gravimetrically (13) Total plasma cholesterol was determined by the method of Runde (30) and triglyceride as glycerol by an enzymatic method (10) commercially available from Boehringer Mannheim. The plasma phospholipid concentration was determined as inorganic phosphorus by the molybdenum blue method (27) in the lipid extract used for total lipid estimation The concentration of phospholipids was expressed as lecithine (phosphorous multiplied by twenty five) (17)

### *Setting normal limits*

Setting normal limits for plasma lipid concentrations certainly gives rise to many difficult considerations of the nature of normality Without doubt normal material selected like ours i e a control group of patients in the same age group as the coronary patient group contains a great number of persons suffering from coronary

Table I *The percentage of under and overweight in the different groups investigated*

| | Underweight | | | Overweight | | |
|---|---|---|---|---|---|---|
| | Men | Women | Total | Men | Women | Total |
| Coronary patient group | 1.3 | 12.9 | 4.6 | 57.1 | 61.3 | 58.3 |
| Hospital control group | 15.4 | 22.2 | 18.2 | 30.8 | 27.8 | 29.5 |
| Young control group | 12.1 | 50.0 | 30.8 | 9.1 | 3.1 | 6.2 |

atherosclerosis not yet manifested as ischaemic heart disease Whether the known increase in plasma lipid concentration in older persons (8) appears as a function of increasing age or means that among older persons there will be an increasing number with *pathological* lipid values (i.e two populations) is not known for sure The low plasma lipid values seen among people with low incidence of atherosclerosis, e g. in rural populations in Asia, seem to point to the latter alternative (31) Consequently we have chosen to define our normal limits for plasma lipid from our young controls, i.e the persons who exhibit the lowest plasma lipid values However a comparison between the coronary patients and the hospital control group has also been made

## RESULTS

*Body weight*

The body weights of the different groups are shown in Table 1 Under or overweight is defined as a weight more than ±10 of an ideal weight referred to height and sex in a Scandinavian population (25)

The three groups differed significantly from each other underweight occurring significantly more commonly in the hospital control group than in the coronary patient group ($\chi^2=7.34$ $p<0.01$) but not significantly more often among the young controls than among the hospital control group ($\chi^2=2.18$ $p<0.2$)

Overweight occurred significantly most often in the coronary patient group compared with the hospital control group both as a whole ($\chi^2=10.36$ $p<0.0025$) and when compared according to sex (male $\chi^2=5.41$ $p<0.025$ female $\chi^2=5.12$ $p<0.025$) The number of overweight persons among the young controls was so small as to preclude any statistical calculation

*Blood pressure*

Hypertension was defined as diastolic blood pressure exceeding 110 mm Hg measured in supine position after resting for 5–10 min Hypertension was found exclusively in the coronary patient group in two men and four women but the small number of hypertensive patients did not allow any statistical calculation

*Fasting blood sugar levels*

The number of persons with fasting blood glucose values exceeding 100 mg per 100 ml was rather small Such values were found in 13° of the coronary patients similarly in male and female

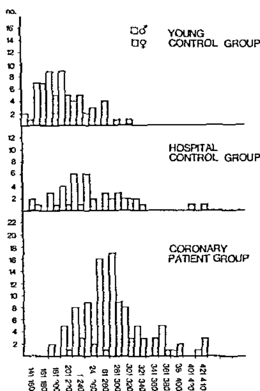



*Fig 2* Plasma cholesterol concentration. Abscissa value in mg per 100 ml.

patients but only in one person in the two control groups

This difference is statistically significant ($p<0.01$ Fischer's exact test)

*Plasma lipid investigations*

The distribution of plasma cholesterol triglyceride phospholipid and total lipid is shown in Figs 2 3 4 and 5

Mean values and statistical calculations (*t* tests) are shown in Table II.

Statistically significant differences between the two sexes occurred in the young control group with higher values among the women for cholesterol phospholipid and total lipid and a tendency not significant, for higher triglyceride values among the men

From the observed values normal upper limits (M ± 2 S D ) were calculated as follows Triglyceride <160 mg per 100 ml for both sexes. Cholesterol male <250 mg per 100 ml female <300 mg per 100 ml Phospholipid male ⩽215 mg per 100 ml female ⩽275 mg per

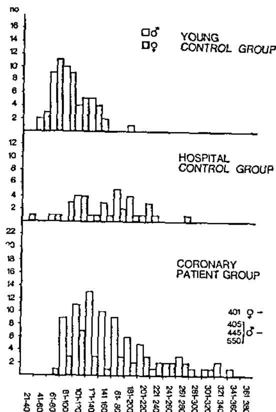



*Fig 3* Plasma triglyceride concentration Abscissa value in mg per 100 ml

ml Total lipid male ⩽675 mg per 100 ml female ⩽790 mg per 100 ml

Hyperlipidaemia i e one of the lipid parameters exceeding the upper normal limit given above was found significantly more commonly in the coronary patient group (80 6 %) than in the hospital control group (54 5 %) also when the sexes were compared in isolation

46 3 % of the coronary patients and 43 2 % of the hospital control group had hypertriglyceridaemia Hypocholesterolaemia was found in 68 5 % of the coronary patients and in 27 3 % of the hospital controls (32 4 and 20 4 % respectively had plasma cholesterol values above 300 mg per 100 ml) 38 9 % of the coronary patient group and 22 7 % of the hospital control group had hyperphospholipidaemia

Both hypertriglyceridaemia and hypercholesterolaemia were found in 35 2 % of the coronary patient group and in 18 2 % of the hospital control group this difference is significant ($p < 0.05$)

The described sex differences in the plasma lipid pattern of the young controls were found in the hospital control group too but to a lesser degree and with no statistical significance However the same sex difference was found in the coronary patient group as in the young controls namely significantly higher plasma cholesterol phospholipid and total lipid concentrations among the women

The result of the chemical lipid assays of the three groups was that the coronary patient group exhibited significantly increased concentration of plasma cholesterol triglyceride and phospholipid, as well as total plasma lipid as compared with both the hospital control group and the young controls Further the hospital control group had significantly higher lipid concentrations (for all fractions) as compared with the young controls. Finally the differences described were found to be significant both when the groups were compared irrespective of sex and when the comparisons were carried out in respect of sex There were two exceptions the difference between the levels of triglycerides and phospholipids in females of the coronary patient group and those of the females of the hospital control group was not

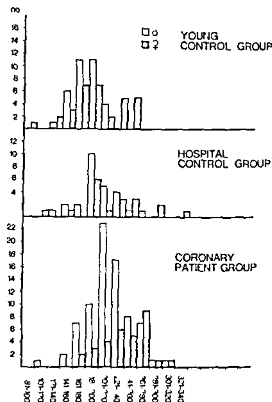



*Fig 4* Plasma phospholipid concentration. Abscissa value in mg per 100 ml

significant, nor was it significant for the phospho lipids of the females in the hospital control group compared with the female young controls

### *Types of hyperlipidaemia*

When hyperlipidaemia was observed the patients were grouped according to the type of hyperlip aemia as suggested by Fredrickson and Lees (14) based on paper lipoprotein electrophoresis

The results together with statistical calculations ($\chi$ test) are shown in Table III

Statistically significant differences between the coronary patient group and the hospital control group were only seen in type II hyperlipoprotein aemia (hyper $\beta$ lipoproteinaemia) (42 6 and 15 9 % respectively) with a tendency to be core common among the men than among the women

Type IV hyperlipoproteinaemia (hyper pre $\beta$ lipoproteinaemia) was found less often in the coronary patient group (15 7 %) than in the hospi tal control group (25 0 %) This difference was not significant for the group as a whole but when the men were compared separately significance was found

When all the groups with elevated pre $\beta$ lipo proteins (Fredrickson s type III IV V) were grouped together and the hospital control and the coronary patient groups were compared no difference was observed

In the hospital control group type II hyperlipo proteinaemia was less common (15 9 %) than types III IV and V together (34 1 %) The contrary was observed among the men in the coronary patient group (46 8 and 32 5 respec tively)

### *Dietary investigations and body weight*

All the persons in the coronary patient group were asked whether they knew of any possible connec tion between ischaemic heart disease and diet and if so did they alter their diet as a consequence of this Based exclusively thereupon they were sepa rated into two groups called coronary diet and no diet Of 28 persons on a coronary diet (25 9 % of the coronary patient group) 22 (78 6% ) had hyperlipidaemia Sixty five (81 3 %) of the rest of the group (80 persons) had hyper lipidaemia There was no significant difference in the frequency of overweight in the two groups

The distribution of men and women in the two dietary groups was nearly equal ( coronary diet

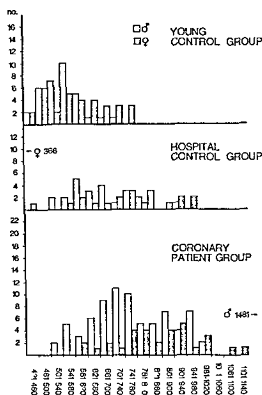



*Fig 5* Plasma total lipid concentration Abscissa value in mg per 100 ml

men/women 2 1 no diet 2 6) The plasma lipid values correlated to diet are given in Table IV The cholesterol and phospholipid values in the two groups showed no differences The higher tri glyceride value in the no diet group was not significantly different from that of the diet group

### *Plasma lipid values related to weight groups*

The mean values for plasma cholesterol triglycer ides and phospholipids are given in Table V The only parameter which shows any difference—not significant however—is plasma triglycerides with higher values among the overweight patients both in the coronary patient group and in the hospital control group The mean value of plasma triglyceride for the overweight hospital controls is exactly the same as that of non-overweight coro nary patients When the overweight patients from both groups were compared with those of normal weight, the difference between the plasma tri glyceride values (182 and 150 mg per 100 ml respectively) was significant ($p < 0.01$)

Table II *Results of chemical plasma lipid assays*

| | Young control group (N) | | Hospital control group (H) | | Coronary patient group (C) | |
|---|---|---|---|---|---|---|
| | 65<br>♂ 33 ♀ 32 | Significance | 44<br>♂ 26 ♀ 18 | Significance | 108<br>♂ 77 ♀ 31 | Significance |
| Total lipid | 574±168<br>550±126 599±192 | $p<0$ 02 | 703±278<br>689±216 725±344 | $p<0$ 50 | 791±[illegible]96<br>771±300 842±[illegible]62 | $p<0$ 01 |
| Cholesterol | [illegible]07±72<br>197±52 218±84 | $p<0$ 0[illegible] | 256±116<br>244±86 272±144 | $p<0$ [illegible]0 | 290±106<br>[illegible]80±92 315±1 0 | $p<0$ 0[illegible] |
| Triglyceride | 92±56<br>97±62 88±50 | $p<0$ 20 | 143±104<br>144±88 140±126 | $p<0$ 80 | 176±168<br>176±172 174±156 | $p$ 0[illegible] |
| Phospholipid | 185±64<br>176±40 193±80 | $p<0$ 05 | 209±82<br>201±62 221±100 | $p<0$ [illegible]0 | 224± 0<br>218±66 239±70 | $p<0$ 0[illegible] |

Mean values and standard deviations for plasma lipid values in the three groups Statistical calculations for differences betw the groups are given In the three first columns the sex differences within the groups are examined In the three last colum comparisons are made between the three groups both as a whole and after dividing them according to sex Values in mg p 100 ml plasma ± 2 s D

The occurrence of type IV hyperlipoproteinaemia as well as the occurrence of pre $\beta$ bands on the lipoprotein electrophoretic strip were uniformly most common among the overweight patients as compared with the patients of normal weight, but in no case was the difference signifi (Table V)

DISCUSSION

The values for plasma cholesterol and phospholipids in the young control group were normally distributed when submitted to probit analyses However this was not the case with the plasma triglycerides of this group Our normal limits for plasma triglycerides are however in good accordance with others based on comparable populations (8) Some investigators believe on the basis of studies of foreign populations (young Africans) (2) that the upper limit for fasting plasma triglycerides of 160 mg per 100 ml as used in this work, is far too high and suggest that the normal upper limit should be about 115 mg per 100 ml and that those with higher values belong to a population biochemically indistinguishable from ischaemic heart patients. Friedman et al (15) have examined a small group of young persons with mild hyper pre beta lipoproteinaemia (i.e detectable pre beta bands on paper lipoprotein electrophoresis) and found that they b haved biochemically differently as compared with controls in their response to intravenous administration of glucose and fructose

The young controls without detectable pre $\beta$ bands in our series (38 persons) had a mean plasma triglyceride value of 85 mg per 100 ml and those with detectable pre $\beta$ bands (27 persons) 103 mg per 100 ml This difference is statistically significant ($p<0$ 02)

Probit analyses of the plasma triglyceride values in two groups (i e with and without pre $\beta$ bands) seem to show two distinguishable normally distributed populations and this fact supports the suggestion of two different populations as mentioned by other investigators (2 15)

When plasma triglyceride values of the two other groups (hospital control group and coronary patient group) are divided according to occurrence of pre $\beta$ lipoproteins the same pattern (two normally distributed populations in each group) was observed but whereas the coronary patients with detectable pre $\beta$ bands when compared with the hospital controls with detectable pre $\beta$ bands, had a significantly higher triglyceride concentration ($p<0$ 005) no significant difference existed between the coronary patients and the hospital controls when the groups without pre $\beta$ bands were compared (Table VI)

On this basis we might conclude that in th multifactorial causality of atherogenesis the lipid aspect could be divided along the following lines.

An age-dependent increasing concentration of

| *H N* | | | *C-H* | | | *C-N* | | |
|---|---|---|---|---|---|---|---|---|
| *H N* | *H♂-N♂* | *H♀-H♀* | *C-H* | *C♂-H♂* | *C♀-H♀* | *C-N* | *C♂-N♂* | *C♀-N♀* |
| p<0 001 | p<0 001 | p<0 01 | p<0 001 | p<0 005 | p<0 02 | p<0 001 | p<0 001 | p<0 001 |
| p<0 001 | p<0 001 | p<0 01 | p<0 001 | p<0 001 | p<0 05 | p<0 001 | p<0 001 | p<0 001 |
| p<0 001 | p<0 001 | p<0 005 | p<0 005 | p<0 02 | p<0 20 | p<0 001 | p<0 001 | p<0 001 |
| p<0 005 | p<0 001 | p<0 1 | p<0 05 | p<0 02 | p<0 [illegible]0 | p<0 001 | p<0 001 | p<0 001 |

all three main plasma lipid fractions is found in Western populations and the highest values of all *three fractions are found among patients with* ischaemic heart disease but whereas the plasma cholesterol and phospholipid values exhibit a nor*mal distribution in young healthy persons and* in older persons without and with coronary athero sclerosis and the age variation therefore could be due to common processes (i e the physiology of ageing —common environment stress faulty diet etc ) the problem of triglyceride metabolism appears somewhat different It seems as if we are already able among young healthy persons to select a biochemically different population with tendency to hypertriglyceridaemia based on the occurrence of a pre $\beta$ band in lipoprotein electrophoresis Later *in life this population is found to* be represented in its severe forms esp cially among patients with ischaemic heart disease

The *nature of this suggested* biochemical defect can of course not be elucidated by an investiga tion like the present one but we think it indicates a more differentiated approach to future dietary prophylactic and therapeutic trials with the aim of preventing coronary atherosclerosis and maybe also throws some light on the fact that previous dietary trials have given varying results (23 29) We think that one of the main reasons for this might be that the group of patients with ischaemic heart disease is too heterogeneous to be submitted to a common dietary restriction The dietary restrictions as followed by 26 % of our coronary patient group did not seem to have any influence *on the plasma lipid levels Most of the dietary* restrictions were rather badly defined but the basic pattern consisted in restrictions of fatty *food and vegetable oil as main fat resource*

The types of hyperlipoproteinaemia found in the coronary patient group may be divided into three main types one with elevated beta lipoproteins (Type II) one with elevation of beta and pre $\beta$ lipoproteins (Type III) and one with elevated pre $\beta$ lipoproteins (Type IV and V) Whereas the first type in the coronary patient group differs both in *frequency and in severity from the hos* pital control group the two other types differed only in severity from the controls This difference *could be due to a different relation in time of the* biochemical defect and the resulting pathoanatomi cal process but a modifying influence of other metabolic processes cannot be excluded The ob served sex difference—the first type being most common among the men and the two other types among the women—seems to point to the latter possibility

The conclusion is however that dietary pro phylactic restrictions should be divided according to these three main types otherwise completely misleading results may be obtained

Electrophoretic lipoprotein assay is a tool with good possibilities as a routine procedure for separating the various plasma lipoprotein abnor

Table III *The distribution of the types of hyperlipidaemia and statistical comparisons between the groups*

| | Young control group (N) | | | | Hospital control group (H) | | | | Coronary patient group (C) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total 65 | ♂ 33 | ♀ 32 | Significance | Total 44 | ♂ 26 | ♀ 18 | Significance | Total 108 | ♂ 77 | ♀ 31 | Significance |
| Type I | 0 | 0 | 0 | | 1 (2 3) | 1 (3 8) | 0 | | 1 (0 9) | 0 | 1 (3 2) | |
| Type II | 1 (1 5) | 0 | 1 (3 1) | | 7 (15 9) | 4 (15 4) | 3 (16 7) | | 46 (42 6) | 36 (46 8) | 10 (32 3) | – |
| Type III | 0 | 0 | 0 | | 4 (9 1) | 3 (11 5) | 1 (5 6) | | 12 (11 1) | 11 (14 3) | 1 (3 2) | |
| Type IV | 0 | 0 | 0 | | 11 (25 0) | 7 (26 9) | 4 (22 2) | – | 17 (15 7) | 7 (9 1) | 10 (32 3) | + $p<0\ 005$ |
| Type V | 1 (1 5) | 1 (3 3) | 0 | | 0 | 0 | 0 | | 9 (8 3) | 7 (9 1) | 2 (6 5) | – |
| Types III–IV–V | 1 (1 5) | 1 (3 3) | 0 | | 15 (34 1) | 10 (38 5) | 5 (27 8) | – | 38 (35 2) | 25 (32 5) | 13 (41 9) | – |
| Not typable | 0 | 0 | 0 | | 1 (2 3) | 0 | 1 (5 6) | | 2 (1 9) | 2 (2 6) | 0 | |
| Detectable pre $\beta$ | 27 (41 5) | 16 (48 5) | 11 (34 4) | – | 30 (68 2) | 19 (73 1) | 11 (61 1) | – | 67 (62.0) | 48 (62 3) | 19 (61 3) | – |
| Hyperlip total | 2 (3 1) | 1 (3 3) | 1 (3 1) | | 24 (54 5) | 15 (57 7) | 9 (50 0) | – | 87 (80 6) | 63 (81 8) | 24 (77 4) | – |

Value not in brackets indicates the absolute number value in brackets the value in per cent
+ = significant difference − = no significant difference

| H N | | | C-H | | | C-N | | |
|---|---|---|---|---|---|---|---|---|
| H N | H♂-N♂ | H♀-N♀ | C-H | C♂-H♂ | C♀-H♀ | C-N | C♂-N♂ | C♀-N♀ |
| | | | + p<0 005 | + p<0 01 | | | | |
| | | | – | | | | | |
| | | | – | + p<0 025 | – | | | |
| | | | – | – | – | | | |
| + <0 01 | – (p<0 10) | – (p<0 10) | – | – | – | + p<0 01 | – | + p<0 05 |
| | | | + p<0 005 | + p<0 025 | + p<0 05 | | | |

Table IV *Plasma lipid values in the coronary patient group referring to possible dietary restriction*

Values in mg per 100 ml ± S D

| | Coronary diet | No diet | Significance |
|---|---|---|---|
| Cholesterol | 290±61 | 290±50 | — |
| Triglyceride | 158±59 | 181±89 | ~$p$<0 10 |
| Phospholipid | 218±34 | 225±37 | — |
| Total lipid | 766±1ˏ8 | 800±154 | ~$p$<0 15 |

Table V *Plasma lipid values related to weight groups*

Values in mg per 100 ml plasma ± S D
None of the differences were significant

| | Hospital control group | | Coronary patient group | |
|---|---|---|---|---|
| | Over weight | Normal and under weight | Over weight | Normal and under weight |
| Cholesterol | 254±54 | 255±61 | ˏ91±56 | 285±47 |
| Triglyceride | 160±47 | 135±53 | 187±85 | 160±78 |
| Phospholipid | ˏ01±32 | 212±44 | 224±37 | 222±36 |
| Type IV | | | | |
| No | 5 of 13 | 6 of 31 | 13 of 63 | 4 of 45 |
| Per cent | 38 5 | 19 4 | 20 6 | 8 9 |
| | 10 of 13 | 20 of 31 | 40 of 63 | 27 of 45 |
| Per cent | 76 9 | 64 5 | 63 5 | 60 0 |

Table VI *Mean triglyceride values (mg per 100 ml plasma)* ± S D *in the three groups when divided according to occurrence of pre β bands in lipoprotein electrophoresis*

| | With pre β bands | ←—— Significance ——→ | Without pre β bands |
|---|---|---|---|
| Young control group | 103±29 | +$p$<0 02 | 85±25 |
| ↕ Significance ↕ | +$p$<0 001 | | +$p$<0 02 |
| Hospital control group | 154±50 | +$p$<0 025 | 117±47 |
| ↕ Significance ↕ | +$p$<0 005 | | – |
| Coronary patient group | 203±45 | +$p$<0 001 | 131±45 |

malities On the other hand a more quantitative approach than can be obtained by the ordinarily used paper lipoprotein technique would be preferable Other media (agarose gel cellulose acetate) seem to be superior to paper electrophoresis in quantitative work and studies with the aim of setting normal limits for the different lipoprotein fractions in young healthy adults are in progress in this laboratory We think that this must be done in other age groups and different populations in order to obtain a better understanding of the pathogenesis and epidemiology of coronary atherosclerosis

ACKNOWLEDGEMENT

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REFERENCES

1 Albrink, M J Triglycerides, lipoproteins and coronary artery disease Arch intern Med 109 345 1962.
Antonis A. & Bersohn I Serum triglyceride levels in South African Europeans and Bantu and in ischaemic heart disease Lancet 1 998 1960
3 Besterman E M M Lipoproteins in coronary artery disease Brit Heart J 19 503 1957
4 Biorck G Blomqvist G & Sievers, J Acta med. scand 156 493 1957
5 Bjorntorp P & Malmcrona, R Serum cholesterol in patients with myocardial infarction in younger ages Acta med. scand. 168 151 1960
6 Blankenhoorn D H Chin, H P & Lau F Y K Ischemic heart disease in young adults Ann. intern. Med 69 21 1968
7 Carlson, L A Serum lipids in men with myocardial infarction Acta med scand. 167 399 1960
8 Carlson L. A. & Lindstedt S The Stockholm prospective study I The initial values for plasma lipids. Acta med scand Suppl 493 1968
9 Dodds, C & Mills G L. Influence of myocardial infarction on plasma lipoprotein concentration Lancet 1 1160 1959
10 Eggstein M & Kreutz, F H Eine neue Bestimmung der Neutralfette im Blutserum und Gewebe Klin. Wschr 44 262, 1966
11 Enger S C & Ritland, S Serumlipider ved hjerteinfarct, preliminær meddelelse Nord med. 81 184 1969
12 Epstein F H Hyperglycemia. A risk factor in coronary heart disease Circulation 36 609 1967
13 Folch, J Lees, M & Sloane Stanley G H A simple method for the isolation and purification of total lipids from animal tissues. J biol. Chem. 2ˏ6 497 1957
14 Fredrickson, D S & Lees, R. S A system for phenotyping hyperlipoproteinemia Circulation 35 3 1 1965
15 Friedman, M., Rosenman, R. H. & Sanford, O B.. Response of hyperlipemic subjects to carbohydrates, pancreatic hormones and prolonged fasting. J clin. Endocr ˏ8 1773 1968
16 Gertler M & White P D Coronary heart disease in young adults. Harvard University Press, Cambridge Mass, 1954

17 Hallgren B Stenhagen S., Svanborg, A. & Svennerholm, L. Gas chromatographic analysis of the fatty acid composition of the plasma lipid in normal and diabetic subjects. J clin. Invest 39 14 4 1960
18 Harlan W R. Graham, J B & Estes, E. H Familial hypercho esterolemia a genetic and metabolic study Medicine 45 77 1966
19 Hellstrom, R. Serum lipids in male patients hospitalized for myocardial infarction. Acta med. scand. 18 7_7 1967
20 Jencks, W P & Durrum, E. L. Paper electrophoresis as a quantitative method The staining of serum lipoproteins. J clin Invest 39 1437 1955
21 Kuo P T Hyperglyceridemia in coronary artery disease and its management. J Amer med. Assoc _01 87 1967
2_ Lees, R. S & Hatch, F T Sharper separation of lipoprotein species by paper electrophoresis in albumin containing buffer J lab clin med. 61 518 1963
23 Leren P Virkningen af cholesterolsenkende diett hos men som har gjennemgått hjerteinfarct. Nord. med. 77 658 1967
_4 Lindgren, F T & Gofman J W The ro'e of lipoproteins in coronary disease Bull. schweiz. Akad. med. Wiss. 13 15_, 1957
25 Natvig, H Landsforeningen for Kosthold og helse Kristiansen & Wøien, Oslo 1959
_6. Nestel, P J. Carbohydrate induced hypertriglyceridemia and glucose utilization in ischemic heart disease Metabolism 15 787 1966
_7 Official and Tentative Methods of the American Oil Chemist Society Ca 12–55 American Oil Chemist Society Chicago Ill 1968
_8 Ostrander L D Beverly J N., Block, W D., Francis, T & Epstein F H Hyperglycemia and hypertriglyceridemia among persons with coronary heart disease Ann. intern. Med. 67 34 1967
29 Report of a Research Committee to the Medical Research Council Controlled trial of soya-bean oil in myocardial infarction Lancet _ 693 1968
30 Runde I A standardized direct method for total cholesterol determination in serum with a combined reagent. Scand J clin. Lab Invest. 18 461 1966
31 Shaper A. G Serum-cholesterol diet, and coronary heart-disease Lancet 1 534 1959
32 Tibblin, G & Cramer K. Serum l pids during the course of an acute myocardial infarction and one year after Acta med. scand. 174 451 1963

# A double-blind cross-over comparison of APTIN® (alprenolol) and pentanitrol in angina pectoris

Aubert, A , Nyberg, G , Slaastad, R & Tjeldflaat, L Brit Med J 1 203, 1970

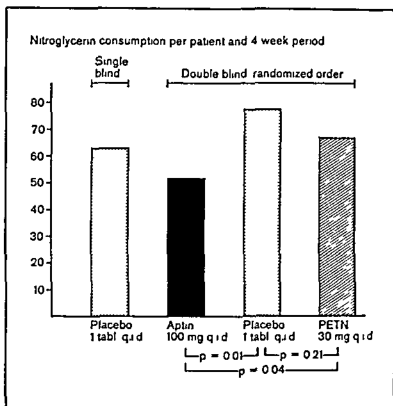
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# SERUM LIPOPROTEIN PATTERN IN MYOCARDIAL INFARCTION

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*Abstract* Normal serum lipids (cholesterol <325 mg/100 ml 8 4 mmol/l and triglycerides <150 mg/100 ml 1 85 mmol/l) were demonstrated during stay in hospital and after three months in 51 of 85 men and 35% of 20 women who survived their first myocardial infarction The hyperlipoproteinaemia was moderate in most of the others Hyper $\beta$ lipoproteinaemia (type II pattern) was found in 22 and hyper pre $\beta$ lipoproteinameia (type IV) in 27 of the men The patients, especially those with type IV had a lower lipid concentration during the acute phase of the infarction than after three months Glucose tolerance was examined in the type IV patients and found to be slightly impaired in 68% of the men

A raised serum cholesterol is connected with increased morbidity from ischaemic heart disease Cholesterol is present in serum combined with triglycerides and phospholipids bound to proteins as lipoproteins (chylomicrons $\beta$ pre $\beta$ and $\alpha$ lipoproteins)

An increased concentration of chylomicrons $\beta$ and/or pre $\beta$ lipoproteins is seen both as primary hereditary conditions and secondary to various diseases Fredrickson et al (6) divided primary hyperlipoproteinaemia into five types and this classification seems suitable for further investigations of the connection between hyperlipoproteinaemia and ischaemic heart disease These authors demonstrated an increased tendency to atherosclerotic diseases in all their types of hyperlipoproteinaemia except type I (chylomicronaemia fat induced hyperlipoproteinaemia")

The commonest types of hyperlipoproteinaemia are type II and type IV Type II is characterized by an increased concentration of cholesterol rich $\beta$ lipoproteins (familial hypercholesterolaemia with xanthomatosis) Type IV patients have an increased serum concentration of pre $\beta$ lipoproteins which also contain cholesterol but especially contain large amounts of triglycerides ( carbohydrate inducible hyperlipaemia )

Endogenous production of pre $\beta$ lipoproteins (type IV pattern) can be induced in normal individuals by a high intake of carbohydrate but is seen in pathological conditions on a normal diet Also an alimentary increase of $\beta$ lipoproteins (type II pattern) may occur on a diet high in cholesterol and saturated fats (16)

Type III (increased $\beta$ and pre $\beta$ lipoproteins) and type V (increased chylomicrons and pre $\beta$ lipoproteins) are uncommon (6) A reduced glucose tolerance is often demonstrated in patients with increased $\beta$ and esp cially pre $\beta$ lipoproteins (3 6 7 9)

No investigations have been published in Norway showing the incidence of the various types of hyperlipoproteinaemia in patients with ischaemic heart diseases We have therefore examined the incidence and distribution of the different lipoprotein patterns in patients with myocardial infarction

## MATERIAL AND METHODS

The occurrence of hyperlipoproteinaemia was investigated in 105 patients below the age of 70 years who had had their first myocardial infarction

The patients were treated in the Medical Department between June 1967 and January 1969 The usual criteria (20) were applied for the diagnosis of myocardial infarction Patients with liver kidney or endocrine diseases apart from diabetes mellitus were excluded Patients with previous myocardial infarction were also excluded in order to limit the number of patients us ng a diet influencing the serum lipids The patients were not recommended any special diet on discharge from hospital apart from weight eduction if necessary Some dietary precautions (usually soyaoil margarine and moderate reduction of saturated fats) had been taken before the infarction by eight

Table I The number of patients in each group the incidence of angina pectoris other cardiovascular diseases and obesity

| | Normal | | Type II | | Type IV | |
|---|---|---|---|---|---|---|
| | Men | Women | Men | Women | Men | Women |
| Number | 43 | 7 | 19 | 8 | 23 | 5 |
| Angina pectoris > 1 month | 14 | 6 | 10 | 5 | 12 | 3 |
| Other atherosclerotic diseases | 4 | 0 | 2 | 0 | 3 | 0 |
| Hypertension | 5 | 0 | 2 | 1 | 0 | 0 |
| 10–19 overweight | 12 | 1 | 4 | 4 | 5 | 0 |
| > 20 overweight | 5 | 2 | 3 | 2 | 6 | 2 |

mined. This was later discontinued because the preliminary results (5) showed that the determination of phospholipids did not contribute further to the demonstration or quantitative estimation of the different types of lipoprotein patterns.

The patients were classified into three groups according to serum lipid values and the lipoprotein electrophoresis pattern normal Fredrickson & Lees's type II and type IV

*Normal* cholesterol ⩽ 325 mg/100 ml and triglycerides ⩽ 150 mg/100 ml in all tests.

*Type II (hyper β lipoproteinaemia)* cholesterol > 325 mg/100 ml in at least one test and at the same time increased β lipoprotein on electrophoresis.

*Type IV (hyper pre β lipoproteinaemia)* triglycerides > 150 mg/100 ml in at least one test and occurrence of pre β lipoprotein on electrophoresis without definite simultaneous increase of β lipoprotein.

patients, after by 29 (i e 20% of normals and type II 45 % of type IV) One patient with type II and one with type IV had xanthomas, and two patients with type IV had xanthelasmas. Patients with arcus corneae and 64 smokers were evenly distributed in the groups. Table I shows the incidence of other cardiovascular diseases and of obesity Anticoagulant therapy was discontinued on discharge from hospital

The serum lipids were investigated in a fasting state on about the 2nd and 9th day after admission and about three months after the infarction. Patients who died before the 9th day were not included. Eight patients died later and one moved away from the district during the observation period. Serum cholesterol (Liebermann Burchard reaction a.m. (23)) and triglycerides (13) were determined, and plasma lipoprotein electrophoresis on paper (14) was carried out

In 32 out of 39 patients with triglycerides > 150 mg/100 ml an oral glucose tolerance test was also carried out (o-toluidine method a.m. (10)) The patients received 1 g glucose kg body weight without previous standard diet. The curve was regarded as pathological when the maximum value was > 200 mg 100 ml or the value at 2 hours was > 140 mg/100 ml at the same time as the 2 ½ hour value was > 120 mg 100 ml.

*The laboratory methods were controlled with known sera.* The cholesterol and glucose tests gave results within the recommended values for Seronorm Nyco

In the first 50 patients phospholipids were also deter-

## RESULTS

Eighty five men and 20 women were studied Table II shows the number of cases percentage distribution and mean age Table III the mean value and standard deviation of cholesterol and triglycerides in men and women in the three groups

*Fig 1 illustrates the mean values* at each of the three separate tests in the men the values and standard deviation are shown in Table IV The women with type IV pattern had lower triglyceride concentration at all the tests but otherwise there was little difference between the mean values in men and women

One man with tuberous xanthoma diverged from the other type IV patients. He had cholesterol 752 mg/100 ml and triglycerides 2238 mg/100 ml after three months, when he had reduced his fat intake The mean and standard deviation of cholesterol and triglycerides (average and after three months) are also calculated for type IV men omitting this patient (Tables III and IV)

Eight of the 19 men and three of the eight women type II pattern had raised triglycerides

Table II Number percentage and mean age of the patients in each group

| | Normal | | Type II | | Type IV | |
|---|---|---|---|---|---|---|
| | Men | Women | Men | Women | Men | Women |
| Number | 43 (51%) | 7 (35 ) | 19 (22 ) | 8 (40%) | 23 (27 ) | 5 (25 %) |
| Mean age (y ) | 59 | 64 | 55 | 63 | 58 | 60 |

Table III *The mean and standard deviation of all the cholesterol and triglyceride estimations in the different groups*

| | Cholesterol (mg/100 ml) | | Triglyceride (mg/100 ml) | |
|---|---|---|---|---|
| | Men | Women | Men | Women |
| Normal | 242 (S D 27) | 244 (S D 34) | 90 (S D 19) | 103 (S D 0) |
| *Type II* | *320 (S D 40)* | *330 (S D 44)* | *117 (S D 32)* | *112 (S D 26)* |
| Type IV | 296 (S D 68)<br>286[a] (S D 43) | 294 (S D 38) | 207 (S D 235)<br>173 (S D 31) | 153 (S D 15) |

[a] Values after omitting one patient see Table IV

(151–196 mg/100 ml) In the type IV group ten of 23 men and two of five women had one or more cholesterol values above 325 mg/100 ml Only four men had triglycerides above 300 mg/100 ml

Slightly reduced glucose tolerance was demonstrated in 14 of 21 men and one of four women with a type IV pattern and in one of the men diabetes mellitus was previously recognized Two patients in the normal group and two in the type II group had non insulin requiring diabetes mellitus Five other type II patients with raised triglycerides had normal glucose tolerance

Ultracentrifuging was not carried out it was therefore not possible to distinguish eventual patients with a type III pattern (6) Types I and V were not seen among the cases investigated

## DISCUSSION

Normal serum lipids were found in 51 % of the men and 35 % of the women Our results regarding women are based on the study of only 20 patients and will not be further discussed Among the 85 men type II hyperlipoproteinaemia was demonstrated in 22 % and type IV in 27 % of the patients Pathological glucose tolerance usually slight was found in 68 % of the men with type IV hyperlipoproteinaemia

Our findings are in good agreement with those of Heinle et al (7) who investigated 88 patients with angiographically verified coronary disease and found 49 % normal 30 with type II and 21 % with type IV hyperlipoproteinaemia and a reduced glucose tolerance in 66 % of the patients equally distributed in the three groups

In Sweden Hellstrom (8) and Carlson and Wahlberg (3) and in Great Britain Rifkind et al (19) have investigated men with ischaemic cardiac disease The latter authors also investigated a control group These authors classified the patients according to the cholesterol and triglyceride levels in groups in which either only one or both lipids were increased They used different upper normal limits for cholesterol and triglycerides The normal limits are given in Table V which shows their results compared with the results in men in the present investigation

The frequency of coronary disease is said to increase with increasing level of cholesterol possibly even above 260 mg/100 ml (4 19) If we had used a lower limit for normal cholesterol than 325 mg/100 ml we would have diagnosed more type II patterns If we like Rifkind et al use 260 mg/100 ml cholesterol as the upper limit we find 19 % of the men with normal 45 % with raised cholesterol alone 35 % with both lipids raised and only one patient with raised triglycerides alone

While raised cholesterol is a recognized risk

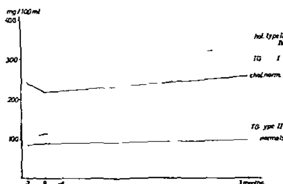



*Fig 1* The variation of the average cholesterol and triglyceride levels in different groups of men during three months after myocardial infarction.

Table IV *The mean and standard deviation of the cholesterol and triglyceride estimations in men on the second and the ninth day and after three months*

| | | Normal | Type II | Type IV |
|---|---|---|---|---|
| Cholesterol (mg/100 ml) | 2nd day | 246 (S D 36) | 314 (S D 60) | 286 (S D 65) |
| | 9th day | 221 (S D 33) | 285 (S D 55) | 65 (S D 39) |
| | 3 months | 261 (S D 28) | 356 (S D 55) | 336 (S D 110) |
| | | | | 316 (S D 61) |
| Triglyceride (mg/100 ml) | 2nd day | 85 (S D 23) | 101 (S D 35) | 156 (S D 64) |
| | 9th day | 90 (S D 34) | 114 (S D 37) | 162 (S D 44) |
| | 3 months | 96 (S D 27) | 131 (S D 33) | 301 (S D 442) |
| | | | | ?09 (S D 99) |

[a] Values after omitting one patient discussed in text with cholesterol 752 mg/100 ml and triglycerides 2238 mg/100 ml after three months

Table V *Percentage of normal and of elevated cholesterol (Chol) triglycerides (TG) or both lipids The authors used different upper normal limits*

| | Normal limit (mg/100 ml) | | | | Elevated | | |
|---|---|---|---|---|---|---|---|
| Authors | Chol | TG | No | Normal ( ) | Chol ( ) | TG ( ) | Chol and TG ( ) |
| Carlson and Wahlberg | 322 | 178 | 100 | 50 | 10 | 25 | 15 |
| Hellstrom | 300 | 150 | 73 | 41 | 15 | ‿0 | 24 |
| Rifkind et al | 260 | 150 | 96 | 43 | 23 | 11 | 23 |
| Enger and Ritland | 325 | 150 | 85 | 51 | 13 | 15 | ‿1 |
| Rifkind et al controls | 260 | 150 | 196 | 78 | 16 | 3 | 3 |

factor in the development of ischaemic cardiac disease (1 2 4 17 18 19 22) the significance of a slight increase of pre $\beta$ lipoprotein and thus triglycerides is more uncertain However several investigations indicate an increased incidence of ischaemic cardiac disease with increasing triglyceride concentration especially in the younger age groups (1 2 3 6 7 8 9 11 12 17 18 19) We found a higher mean age of type IV than type II men

Fredrickson et al (6) and others (11 12) have shown that reduction of carbohydrate intake reduces serum lipids in type IV patients and also that they react well to drug therapy But we still lack controlled clinical investigations showing a reduced morbidity and mortality after reduction of the pre $\beta$ lipoprotein level

In accordance with other investigators (15 21) we found (Fig. 1 Table IV) lower lipid concentrations during the acute phase of the infarction than after three months This was found in the normal group and in type II but it was especially pronounced in type IV And like others we found a high incidence of reduced glucose tolerance in type IV patients

If further investigations show the desirability of a special dietary regime in mild hyper pre $\beta$ lipoproteinaemia it seems important that the serum lipid pattern should be examined after the acute phase of the infarction is over

## REFERENCES


1 Brown, D F., Kinch S H & Doyle J T New Engl J Med 273 947 1965
2 Carlson, L A Acta med. scand. 167 399 1960
3 Carlson, L A & Wahlberg, F Acta med. scand. 180 307 1966
4 Dawber T R., Kannel, W B Revotskie, N & Kagan A Proc roy Soc. Med 55 265 1962
5 Enger S C & Ritland, S Nord. Med. 81 184 1969
6 Fredrickson, D S., Levy R. I & Lees, R. S. New Engl. J Med. 276 34 1967
7 Heinle A. Fredrickson, D S Levy R. I., Herman, M V & Gorlin, R. J clin Invest 46 1069 1967

8 Hellstrom, R. Acta med scand. 182 727 1967
9 Hood, B Örndahl, C., Ahlstrom M. & Angervall, G Acta med. scand 181 671 1967
10 Hultman, E. Nature 183 108 1959
11 Kuo P T J Amer med Ass 01 87 1967
12. — Ann. intern Med. 68 449 1968
13 Laurell S Scand J clin Lab Invest 18 668 1966
14 Lees, R. S & Hatch, F T J Lab clin Med 61 518 1963
15 Leren P J Oslo Cy Hosp 10 55 1960
16 Levy R. I & Fredrickson D S Amer J Cardiol 22 576 1968
17 Mills G L. & Wilkinson, P A Brit. Heart J 28 638 1966
18 Nicolaysen, R & Westlund K. Scand. J clin Lab Invest. 15 291 1963
19 Rifkind, B M., Lawson, D & Gale M J Atheroscler Res. 8 167 1968
20 Ritland, S & Lygren, T Lancet 1 122, 1969
21 Tibblin, G & Cramér K. Acta med scand 174 451 1963
22. Westlund K. & Nicolaysen R. Scand J clin Lab Invest. Suppl 87 1966
23 Zurkowski, P Clin Chem. 10 451 1964

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# DETERMINATION OF DIGITALIS IN BLOOD

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*Abstract* Digitalis glycosides inhibit cation transport across cell membranes By measuring the inhibition of transmembrane transport by digitalis standards the unknown digitalis concentration in patients blood samples can be determined. The original method (by Lowenstein) has been further tested and modified to obtain increased accuracy precision and capacity No significant interference by other preparations than digitalis has been found. The described modification is suitable for routine control of patients using different digitalis glycosides

Digitalis preparations have been used since A D 1785 The dosage has been regulated according to the clinical judgement of the physician The increasing frequency of digitalis intoxications has made the need for better control of digitalis medication imperative

## *Principle*

The high K and low Na concentrations in the cells are maintained by active transmembrane transport The energy for this transport against the concentration gradient is delivered by ATP Cardioactive digitalis glycosides (in the following referred to as digitalis) decrease cation transport by inhibiting a specific enzyme which catalyzes the liberation of the energy (Na K activated ATPase) (8 10 14) Lowenstein has used this phenomenon to determine digitalis concentration in blood (16 17) He measured the transport of cations through the membranes of normal erythrocytes in the presence of digitalis standards or patients sera The determination involves the following steps extraction of digitalis from standards and patients samples—incubation of erythrocytes with extracts—registration of the transmembrane transport that has occurred. The transport of cations is followed with $^{86}$Rb a convenient radioactive isotope which is treated as K by the red blood cells (15 20)

Lowenstein has given few details concerning the optimal conditions of each step The purpose of the present investigation was to improve his method and to test possible non-digitalis inhibitors of Rb transport A modification simpler to perform with greater capacity and which seems to give better precision than the original method is described

## REAGENTS

*$^{86}$Rb solution*

$^{86}$RbCl (1 85 g/100 ml) with specific activity 500 to 50 $\mu$C/mg Rb was obtained from Institutt for Atomenergi, Kjeller Norway The working solution contained 400 $\mu$C $^{86}$Rb in 100 ml NaCl (0 9 g) glucose (0 7 g) solution

*Suspension of erythrocytes*

Human erythrocytes in ACD solution (stored at +4 C and not older than 10 days) were gently washed with ice-cold isotonic NaCl until about 99% of plasma was removed. The erythrocytes were resuspended in isotonic NaCl to obtain 17 5 g (±1 0) hemoglobin/100 ml

*Digitalis standard solution*

Digitoxin (as solution of Digitrin, AB Astra, Sweden) was diluted with distilled water to make 1 0 and 0 1 $\mu$g digitoxin per ml This stock solution was added to pooled plasma (without digitalis) to make 0-50 ng digitoxin per ml.

*Dichloromethane*

Analytical grade specific weight 1 32, boiling point approximately +40°C

*Equipment for measurement of radioactivity*

A liquid scintillation counter is recommended. In several experiments leading to the adopted method a well crystal scintillation counter and a gas proportional counter have also been used

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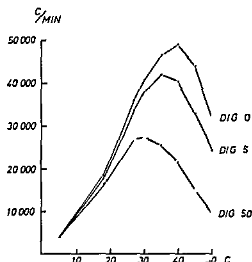



*Fig. 1.* Intracellular Rb transport in the presence of digitoxin 0-5-50 ng at different incubation temperatures. Intracellular radioactivity in counts/min along the vertical axis.

*Patients' blood samples*

Serum or plasma (with EDTA) were used. The samples had been stored at +4 C and the serum/plasma was separated from erythrocytes within two days.

## STANDARD PROCEDURE

*Extraction*

Serum 1.0 ml (or standards) was extracted with 3.0 ml dichloromethane for 5 min in a mixing machine. The two phases separate spontaneously and 2.0 ml dichloromethane was transferred to disposable glass tubes and evaporated in a water bath at +50 C for half an hour.

*Incubation*

The dried extracts were dissolved in 0.25 ml (1.0 $\mu$C) Rb solution and the tubes were placed in ice-cold water for 5 min. One and a half ml cold blood suspension was added with an automatic syringe. The incubation was performed in a water bath at +40 C. After 4 hours the tubes were put back in the ice bath.

*Recording of Rb uptake*

The $^{86}$Rb uptake can be determined in two ways: measurement of the $^{86}$Rb present in the cells or the $^{86}$Rb remaining outside the cells. Intracellular radioactivity was recorded in a well crystal scintillation counter. The extracellular $^{86}$Rb was removed by washing the erythrocytes three times with the tenfold volume of ice cold NaCl. In the recommended procedure the erythrocytes were spun down and the radioactivity in 0.2 ml cell free supernatant was recorded in a liquid scintillation counter.

## RESULTS AND COMMENTS

*The efficiency of the extraction*

Tritiated digoxin (obtained from Burroughs Wellcome & Co.) was diluted with plasma to give 1 $\mu$g digoxin and 0.1 $\mu$C tritium per ml. One ml plasma was extracted with 3 ml dichloromethane by tilting the tubes up and down in a mixing machine with about 20 rotations/min for increasing periods.

Samples of plasma from patients on digitoxin therapy and plasma containing known amounts of added digitoxin were subjected to repeated extractions with three successive portions of dichloromethane. The dichloromethane was evaporated and the digitoxin concentration measured.

Both experiments showed that a single extraction of one min duration gave 95–100% extraction of digoxin and digitoxin from plasma.

The following experiments have all been performed without extraction and with digitoxin added directly into the incubation tubes.

*The influence of the incubation temperature*
(Figs. 1 and 2)

Standard procedure with the exception that the incubation was performed at different temperatures. The uptake of Rb in the cells reaches a maximum at about +40°C when digitoxin is absent; with digitoxin present the optimal temperature is somewhat lower. The discrimination between different concentrations of digitoxin increases with increasing temperature (Fig. 2). However, as temperatures above +40°C lead to increased hemolysis, the incubation temperature of +40°C is chosen. The transmembrane transport

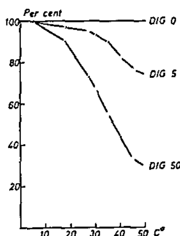



*Fig. 2.* Discrimination between samples containing digitoxin 0-5-50 ng at different incubation temperatures. Intracellular counts/min of samples without digitoxin taken as 100.

is nearly inhibited at low temperatures (21) This phenomenon is applied in the present method to secure a constant incubation time

*The influence of pH during incubation* (Fig 3)

Standard procedure but the pH of the medium was adjusted to different levels by means of TRIS/maleate buffer The intracellular uptake of Rb has a maximum at pH 7 2–7 4 and this value is obtained with the standard procedure (6) The discrimination between different digitoxin concentrations increases in an acid medium but this has little practical value due to the low intracellular uptake

*The influence of the concentration of red blood cells and the incubation time*

Standard procedure with the exception that blood cell concentration and incubation time were varied Without digitalis the uptake of $^{86}$Rb increases with the blood cells present and with the incubation time (Fig 4) With concentrated blood suspensions maximal uptake is obtained after 3–4 hours when nearly 80 of the radioactivity is inside the cells In Fig 5 the blood cell concentration increased while the incubation time was kept constant (4 h) The discrimination increases with the blood cell concentration up to about 15 g Hb/100 ml With higher erythrocyte concentration

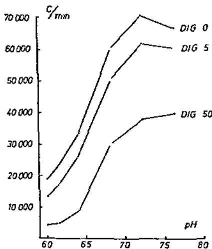



*Fig 3* Intracellular Rb transport in the presence of digitoxin 0–5–50 ng at different pH values of the medium The pH measured with Astrup apparatus (Radiometer) Intracellular radioactivity in counts/min along the vertical axis.

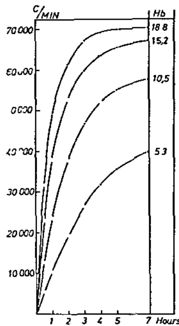



*Fig 4* Intracellular Rb transport as a function of blood cell concentration and incubation time The incubation time along the horisontal axis, and the intracellular radioactivity in counts/min along the vertical axis The blood cell quantity expressed as g Hb/100 ml reaction mixture in the right column.

(21 8 g) a lower discrimination has been found The probable explanation is an increased tendency to hemolysis in concentrated blood suspensions and that the liberated intracellular K is diluted in a corresponding small volume of extracellular fluid The high K concentration decreases the inhibition by digitalis (6 10) When the blood cell concentration was constant (14 9 g Hb/100 ml reaction mixture) the discrimination increased with the incubation time (Fig 6)

These experiments demonstrate that recording of the radioactivity in the cell free supernatant requires a high blood cell concentration and long incubation time Optimal discrimination is obtained with about 15 g Hb/100 ml in the incubation tubes Higher concentrations do not increase the discrimination and such suspensions are too viscous to be pipetted with any precision Incubation for 4 hours gives maximal intracellular Rb uptake and longer periods increase the risk of hemolysis Hemolysis indicates leaky membranes with possible back diffusion of Rb and decreased discrimination between high and low digitalis values

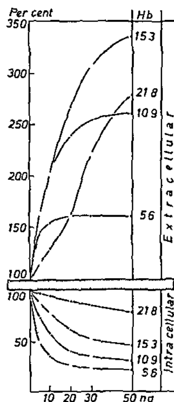



*Fig 5* Discrimination between samples with different amounts of digitoxin at different blood cell concentrations Incubation time 4 h The digitoxin concentration in ng along the horisontal axis Counts/min of the samples digitoxin taken as 100 o The lower part of the figure shows the results of intracellular the upper part the results of extracellular recording of radioactivity Hb concentration in g/100 ml reaction mixture in the right column

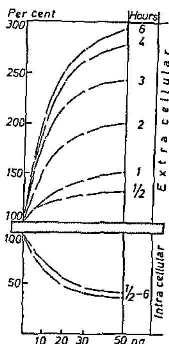



*Fig 6* Discrimination between samples with different amounts of digitoxin at different incubation times Hb concentration 14 9 g/100 ml Incubation time in the right column Otherwise Fig 6 = Fig 5

The recording of intracellular radioactivity shows that the discrimination is nearly constant with short or long incubation (Fig 6) Diluted blood suspensions can be used but give low discrimination when the digitoxin concentration is above 10–20 ng

*Variations in the concentration of cations rubidium – constant specific activity – variable quantity* (Fig 7)

Standard procedure with one exception—increasing amounts of Rb were used The uptake of Rb in the cells increases nearly linearly with the amount of Rb in the extracellular fluid However the discrimination is not improved This means that the Rb dose per sample can be adjusted within a wide range according to the labora

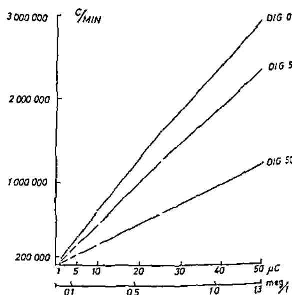



*Fig 7* Intracellular Rb uptake with increasing Rb concentration with constant specific actvity in the extra cellular fluid The quantity of Rb (in μg per sample and in mEq/l in the reaction mixture) is along the horisontal axis The intracellular radioactivity in counts/min along the vertical axis

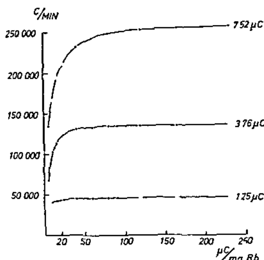



*Fig 8* Intracellular Rb uptake with three different [86]Rb quantities (1.24–3.76–7.52 μC per sample) and with variable specific activity in the extracellular fluid. Specific activity in μC/mg Rb along the horisontal axis. The intracellular radioactivity in counts/min along the vertical axis.

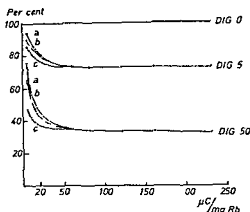



*Fig 9* Discrimination between samples with digitoxin 0–5–50 ng with three different [86]Rb quantities per sample and with variable specific activity in the extracellular fluid. The specific activity in μC/mg Rb along the horisontal axis. Counts/min of the samples without digitoxin taken as 100%. The intracellular radioactivity in per cent along the vertical axis. Curve *a* shows intracellular radioactivity with 7.52 μC per sample, curve *b* 3.76 μC per sample and curve *c* 1.25 μC per sample.

tory's equipment for radioactive recording. In doing this it must be remembered that with high K (or Rb) concentrations (above 4 mEq/l) a slower and non-linear intracellular transport has been found in samples without digitalis (6, 10). With digitalis present a high K or Rb concentration may further decrease the inhibition by digitalis (6, 10).

*Rubidium – variable specific activity – variable quantity*

Standard procedure with the exception that three different Rb solutions were used with 1.25–3.76–7.52 μC per sample. By adding stable Rb the specific activity varied from 232 to 4.0 μC/mg Rb. The total concentration of Rb ranged between 0.04 and 12.83 mEq/l. Fig. 8 demonstrates that the uptake of Rb is independent of the specific activity when this is above 50–60 μC/mg Rb. With lower specific activities (higher concentrations of stable Rb) the stable Rb suppresses the incorporation of radioactive Rb, especially when high Rb doses are used. The discrimination between different digitoxin concentrations decreases when the specific activity is below 50 μC/mg Rb (Fig. 9).

In the standard procedure 1 μC per sample and 0.2 ml supernatant in the liquid scintillation counter give high and reliable counting results. The Rb solution can be used until the specific activity has decreased to about 20 μC/mg Rb.

*Sodium* (Fig. 10)

Red blood cells were washed five times with isotonic choline chloride to remove extracellular Na

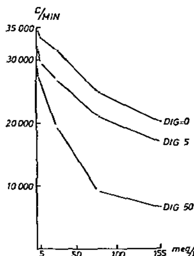



*Fig 10* Intracellular Rb transport in the presence of digitoxin 0–5–50 ng and with variable sodium concentrations in the extracellular fluid. The sodium concentration along the horisontal axis, and the intracellular radioactivity in counts/min along the vertical axis.

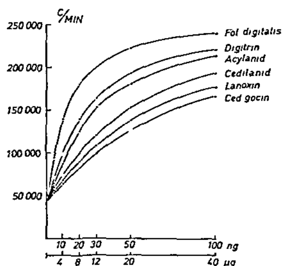



*Fig 11* Standard curves for six different digitalis preparations. For Folium digitalis concentration expressed in μg/ml, all the others in ng/ml Recording of radioactivity done in the cell free supernatant and given as counts/min.

The erythrocytes were incubated with different Na concentrations otherwise standard procedure The uptake of Rb in the cells increases with decreasing concentration of Na (6 7) However this is of little value because the discrimination ecreases with low Na concentrations

*Calcium and magnesium*

Standard procedure with one exception Ca and/or Mg were added to the extracellular fluid The concentrations were 0 025 or 1 025 mEq/l The presence of these cations alone or together was without influence on Rb uptake and digitoxin discrimination This is in accordance with previous experience Mg stimulates and Ca inhibits trans membrane transport, but they work only on the inside surface of the cell membrane (6 8)

*Standard curves*

Typical standard curves for six commercial digitalis preparations are shown in Fig 11 The trade names and producers are presented in Table I The therapeutic digitoxin concentration in serum is about 10–30 ng/ml and in case of intoxication the value may increase to 50–60 ng/ml (1 17 18 19 and own observations) One of the purposes of this investigation has been to obtain good discrimination in the whole 0–60 ng range In most experiments presented the digitalis glycosides have been added directly into the incubation tubes In the standard procedure including extraction only 2/3 of the digitalis in one ml serum is transferred to the incubation tubes This must be remembered when reading the figures In Fig 11 the standard curve for digitoxin is not linear However in the majority of the cases the results will be within the first and more linear portion of the standard curve

With digoxin the therapeutic serum level is about 0–5 ng/ml (9 16 17) The standard curve is strictly linear in the 0–10 ng range and the counts per min of the 10 ng sample are 150% of the sample without digoxin By increasing the serum volume the discrimination may be even better

The other digitalis preparations have their own standard curves but the therapeutic serum levels have not yet been investigated

*Accuracy and precision*

Digitoxin was added to pooled digitalis free plasma to obtain 20 0 ng/ml and the plasma was divided into several tubes and stored at −20 C This control plasma has been run in duplicate 27 times The mean value and standard deviation were 20 35 ± 1 94 ng/ml (101 7 ± 9 5 % ) Serum from patients beginning digitoxin medication or on permanent therapy were analyzed twice on dif

Table I *Digitalis preparations tested with standard curves*

| Generic name | Trade name | Producer |
|---|---|---|
| Folium digitalis | Folium digitalis | Nyegaard & Co Norway |
| Digitoxin | Digitrin | AB Astra Sweden |
| Acetyl-digitoxin | Acylanid | Sandoz, Switzerland |
| Desacetyl lanatoside | Cedilanid | Sandoz, Switzerland |
| Digoxin | Lanoxin | Burroughs Wellcome & Co England |
| Desglucolanatocide C | Cedigocin | Sandoz, Switzerland |

Table II *Precision with repeated analysis of patient samples*

| Digitoxin | 0–10 ng | 11–20 ng | 21–30 ng |
|---|---|---|---|
| No of analyses | 39 | 27 | 15 |
| Mean nanogram | 5 48 | 14 78 | 23 84 |
| S D nanogram | 0 738 | 0 997 | 2 15 |
| Coefficient of variation | 13 5 | 6 6 | 9 0 |

ferent days The precision increases with increasing digitoxin concentration (Table II)

*Specificity*

The inhibition of cation transport was initially regarded as a specific ability of cardioactive digitalis glycosides but during the last years other drugs have been shown to interfere with transmembrane transport The following preparations were tested mersalyl (12) furosemid (13) ethacrynic acid (5) lidocaine (12) promethazine (12) chlorpromazine / perphenazine / prochlorperazine / trifluoperazine (2) The preparations were added directly into the incubation tubes without extraction. The concentrations were calculated as the usual dose of the medicaments diluted in 5 l blood volume With furosemid and ethacrynic acid a slight inhibition of Rb uptake was found corresponding to 1 0–1 5 ng digitoxin With mersalyl a distinct hemolysis of erythrocytes occurred this was due to a pH deviation to 8 3 All the other drugs showed no influence

Control patients not receiving digitoxin were given parenteral mersalyl furosemid or ethacrynic acid Blood samples taken one hour later did not contain inhibiting substances

Insulin and hydrocortisone were also tested in vitro and in vivo but no influence was observed The following drugs have been tested by other authors and are without effect on Na K activated ATPase chlorothiazide, acetazolamide cyclopentazide spironolactone and cortisone (5 8) Two cortisone derivatives (prednisone and prednisolonebisguanylhydrazone) have been found to inhibit Na K activated ATPase this has not been tested (4)

## DISCUSSION

The present theory of transmembrane transport and drug inhibition has recently been reviewed (8 10 14) Cardioactive digitalis glycosides inhibit Na K activated ATPase and the specific group responsible for this effect is the unsaturated lactone ring attached with beta-configuration to C 17 in the steroid molecule (8) The relation between this phenomenon and the therapeutic inotropic cardiac effect is doubtful (8) The inhibition by other drugs may be through other mechanisms (10)

During the years many principles for digitalis determination have been proposed Friedman s duck heart bioassay and similar methods are too laborious for clinical routine determinations and the sensitivity is too low for serum measurements (3) With pure digitalis glycosides different photometric fluorimetric and polarographic determinations can be used but applied to biological samples these methods require initial extractions and purifications Up to the present time the best variant seems to be Jelliffe s method with chromatographic isolation of digitoxin or digoxin from urine (11) The capacity is low one technician one sample one day

Most of our present knowledge of the concentration and metabolism of digitalis has been obtained during the last 15 years by the use of radioactive digitalis tracers (3) These investigations have been extremely valuable but are impossible to apply to repeated clinical use with ordinary heart patients.

Three new methods based on the in vitro use of radioactive isotopes have recently been described Lukas and Peterson use double isotope dilution technique (18) They start with 10–15 ml serum and the procedure is rather laborious If the sample contains 10–20 ng the coefficient of variation is 20% and with 200 ng 4%

Oliver et al have developed an immuno-precipitation method (19) The assay involves binding of [1] I labeled tyrosine digitoxigenin by rabbit antibody Antibody bound radioactivity is precipitated by a second antibody Unlabeled digitoxin from patients can be determined by the extent to which it competes with the labeled digitoxigenin They use 5 ml serum and the coefficient of variation is 32 % in the 0–10 ng range and 11% in the 20–45 ng range The method is at present rather time-consuming (36 hours)

Both these methods measure digitoxin but studies are in progress to develop modifications that can be used with other digitalis glycosides

Burnett and Conklin have recently applied the idea of Lowenstein by a more direct and simple approach (1) They prepared Na K activated ATPase from different tissues added ATP and measured the liberated phosphorus after 15 min incubation. Digitoxin inhibits the enzyme and the liberation of phosphorus This method is technically much simpler than the described procedure but is not sensitive enough to be used for digoxin determination where the therapeutic concentra

tion is in the 0–5 ng range Further it is possible that other drugs (Specificity page 377) may prove to be inhibitors of this system and give false positive results this has not been tested by the authors

The modifications of Lowenstein s method have been introduced to obtain greater capacity and precision Different solutions (especially chloroform) have been used for extraction of digitalis from leafs serum urine stool and homogenized tissues However the most simple and efficient extraction from serum is obtained with dichloromethane The optimal conditions during incubation are demonstrated in Figs 1–10 Registration of the radioactivity in the cell free supernatant with a liquid scintillation counter requires only one centrifugation after incubation With a well crystal scintillation counter the counting yield was much lower With a gas proportional counter the counting yield was about half of that with the liquid scintillation counter and the coefficient of variation was 2–4 times greater Registration of the intracellular radioactivity is more laborious the counting yield is low and the repeated washing procedure may lead to loss of cells and radioactivity

The accuracy and precision of the described compare favorably with other methods sensitivity is sufficient and with digoxin and digitoxin only one ml serum is necessary

The specificity is more difficult to evaluate Theoretically different digitalis glycosides and digitalis mixtures (Folium digitalis) can be measured by using the specific preparation in the standard curve However digitalis glycosides are metabolized in the body and for instance digitoxin is partly transformed to digoxin and eliminated by renal excretion (18) It is possible that some of the inhibitions measured by this method is caused by metabolites in serum If this is true the correct answer of digitalis determination should be X nanogram per ml measured as digitoxin As far as we know at present there is a strong relationship between the inotropic cardioactive effect in vivo and the inhibition of Na K activated ATPase in vitro (8) This means that if the metabolites are more cardioactive than the administered glycoside this will give increased enzyme inhibition and high digitalis values in serum

The relation between the concentration of digitalis in serum and in myocardium has also been questioned Investigations with radioactive isotopes have shown a rather constant proportion with a myocardial concentration that is about 40 times higher than in blood (3) A high serum value is probably a good indicator of high digitalis concentration in myocardium This may—or may not—have clinical importance depending on other known (potassium) and unknown factors

The capacity of the present method is great One technician can handle series up to 200 tubes consisting of 90 patients samples in duplicate and 20 standards and controls The preparation and extraction are performed in one day The incubation and radioactive recording are done on the next day With series of 200 tubes the time spent on each pair of patient samples is about 10 min It is impossible to give an immediate answer in a critical situation Working with short series the preparation takes one hour the incubation (3)–4 hours and the recording of radioactivity one hour

At present the described procedure is useful for physiological studies and for the detection of chronic digitalis intoxication especially among patients with impaired digitalis tolerance and elimination The practical value of an optimal digitalis therapy guided by blood determinations remains to be investigated

## REFERENCES


1 Burnett G H & Conklin R. L The enzymatic assay of plasma digitoxin levels J Lab clin Med. 71 1040 1968
2 Davis P W & Brody T M Inhibition of Na K activated adenosine triphosphatase activity in rat brain by substituted phenothiazines Biochem Pharmacol 15 703 1966
3 Doherty J E The clinical pharmacology of digitalis glycosides A review Amer J med Sci 255 382, 1968
4 Dransfeld H & Greff K Der Einfluss des Prednison und Prednisolonbisguanylhydrazons auf die Na + K -stimulierte Membran ATPase des Meerschweinchenherzens Naynyn Schmiedebergs Arch exp Path Pharmak. 249 425 1964
5 Duggan D E & Noll R. M Effects of ethacrynic acid and cardiac glycosides upon a membrane adenosinetriphosphatase of renal cortex. Arch Biochem. Biophys 109 338 1965
6 Dunham E T & Glynn I M Adenosinetriphosphatase activity and the active movements of alkali metal ions J Physiol 156 274 1961
7 Garrahan P J & Glynn I M The sensitivity of

the sodium pump to external sodium. J Physiol. 197 175 1967

8 Glynn I M The action of cardiac glycosides on ion movements. *Pharmacol. Rev 16 381 1964*

9 Grahame Smith, D G & Everest, M. S Measurement of digoxin in plasma and its use in diagnosis of digoxin intoxication Brit. med. J 1 [illegible]86 1969

10 Hoffman, J F The red cell membrane and the transport of sodium and potassium. Amer J Med. 41 660 1966

11 Jelliffe, R. W A chemical determination of urinary digitoxin and digoxin in man. J Lab clin Med. 67 694 1966

12 Jones, V D., Lockett, G & Landon E. L. A cellular action of mercurial diuretics. J Pharmacol. & exper Ther 147 23 1965

13 Judah, J D Action of antihistamines and other drugs on ion and water movements in cells and mitochondria. Fed. Proc. 21 1097 1962.

14 Katz, A. I. & Epstein, F H Physiologic role of sodium-potassium activated adenosine triphosphatase in the transport of cations across biological membranes. New Engl J Med. 78 [illegible]53 1968

15 Love W D & Burch, G E. A comparison of potassium$^{42}$ rubidium$^{86}$ and cesium$^{134}$ as tracers of potassium in the study of cation metabolism of human erythrocytes in vitro J Lab clin. Invest. 41 351 1953

*16 Lowenstein J M A method for measuring plasma* levels of digitalis glycosides. Circulation 31 228 1965

17 Lowenstein, J M & Corrill, E. M. An improved method for measuring plasma and tissue concentrations of digitalis glycosides. J Lab clin. Med. 67 1048 1966

18 Lukas, D S & Peterson, R. E Double isotope dilution derivative assay of digitoxin in plasma, urine and stool of patients maintained on the drug. J clin Invest. 45 782, 1966

19 Oliver G C., Parker B M., Brasfield, D L & Parker C. W The measurement of digitoxin in human serum by radioimmunoassay J clin Invest. 47 1035 1968

20 Walz, F G & Chan, P C Effects of cysteine and potassium on the ATP-dependent retention of sodium ions by erythrocyte membranes. Biochim. biophys. Acta 135 885 1967

21 Wood, L. & Beutler E. Temperature dependence of sodium-potassium activated erythrocyte adenosine triphosphatase J Lab clin. Med 70 [illegible]87 1967

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# NECROTIC PHAEOCHROMOCYTOMA WITH GASTRIC HAEMORRHAGE SHOCK, AND UNCOMMONLY HIGH CATECHOLAMINE EXCRETION

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*Abst act* A 43 year-old man who was admitted to hospital for gastric haemorrhage had a necrotic phaeochromocytoma. The blood loss was corrected, but the patient died in irreversible shock 4 days after admission. There was adrenergic myocardiopathy The catecholamine excretion was 19,-00 μg/24 h vanilmandelic acid excretion 398 mg/24 h. Values of this magnitude have not been found elsewhere. The autopsy showed necrotic phaeochromocytoma, atrophic gastritis with mucosal necrosis, and myocardiopathy It is suggested that the tumour necrosis took place as a result of malignant growth within a firm capsule Very high catecholamine excretion values may indicate the need for emergency surgery in phaeochromocytoma

The common manifestations of phaeochromocytoma—hypertension postural hypotension palpitation paroxysmal tachycardia decreased intestinal motility sweating and hypermetabolism—are well documented Shock following haemorrhage into the tumour or necrosis of the tumour has been described six times (3 4 6 8 9 10) with fatal outcome in four of these cases (3 6 9 10) In one of the surviving patients the diagnosis was known before the acute episode We do not know of any previous report of phaeochromocytoma with gastric haemorrhage neither has the level of catecholamine excretion been reported before in patients in whom destruction of the tumour caused the acute situation

## CASE REPORT

A 43 year-old man was admitted to hosp tal because of haematemesis.

A brother of the patient had d ed of an unidentified malignant abdominal tumour

In recent years the patient had been healthy During the past two or three months he had had unspecific abdominal distress, moderate constipation and occasionally paroxysmal palpitation and sweating during defaecation. On the day of admission he had epigastric pain and nausea and vomited an unspecified amount of haemorrhagic fluid.

The patient s skin was cold and mo st. On the arms and trunk there were numerous, pea-sized, brownish skin tumours. The pulse rate was 1-0/min, the blood pressure 150 1 0 mm Hg. Infusions of low molecular dextran and electrolytes were begun, and three units of whole blood were given. The blood pressure became stable at 140–1-0 90 mm Hg at a pulse rate of 100–1-0 min. On one occasion the blood pressure suddenly rose to 2-0 mm Hg systolic accompanied by a pulse rate of 1 0 min. The peripheral circulation was continuously impaired the extremities were cold, with bluish nailbeds and slow cap l lary refilling. Subsequently the perspiration became profuse It was found that the blood pressure immediately rose upon palpation of the abdomen and a phaeochromocytoma was suspected. Collection of urine for determination of vanilmandelic acid (VMA) and catecholamines was started

The haemoglobin was 11 0 g 100 ml before and after transfusion. The leukocyte count was 11 700 mm³ with 1% stab neutrophils, 81% segmented neutrophils, 1- lymphocytes and 6% monocytes.

On the second day in hospital the patient becam worse He developed signs of paralytic ileus, the periph eral circulation was grossly impaired and he had haema temesis on two occasions, about 500 ml each Pulmonary rales and a systolic murmur over the apex de eloped and the patient was digitalized. As the probability of phaeochromocytoma was conside ed to be very great, t was now thought necessary to start adrenergic blockade without waiting for the results of the VMA and catechol amine determinations. Before this the laboratory values were checked haemoglobin was 11 0 g/100 ml, the electrolytes, acid-base values and creatinine we e normal Propranolol and phentolamine were then given slowly ntra enously during one hour in total amounts of 7 mg of phentolamine and 3 mg of propranolol. During this trial the blood pressure went down from 170 90 mm Hg

to 110/70 and the pulse rate slowed down from 144/min to 120/min during the first ten minutes Thereafter the blood pressure became very unstable varying between 170 and 85 systolic whereas the pulse rate varied between *144 and 120/min After one hour of blockade the patient* vomited about 1 000 ml of dark red haemorrhagic fluid whereafter the blood pressure fell and the pulse rate rose Whole blood transfusion was started and the blood pressure and pulse rate returned to the values recorded before starting adrenergic blockade The urine output *during the day was 2 100 ml at an intake of 1 800 ml* of fluid with electrolytes and 4 units of whole blood

On the third day the patient s temperature rose to 38 5 C His abdomen became tense and meteoristic perspiration was severe Gastric aspiration and constant duodenal suction drainage yielded 2 500 ml of grossly haemorrhagic fluid The haematocrit was 49 and the elecerolyte and acid base values normal After infusion of 4 000 ml of buffered electrolyte solution the haemato crit was 44 and the output of urine 1 100 ml during the day

The *electrocardiogram* showed left atrial train and variations in the PQ time and QRS voltage The QT time was prolonged and there were ST depressions in all leads

On the fourth day a slight compensated metabolic acidosis developed Serum creatinine was 2 3 mg/100 ml the sodium and potassium values were normal The haema tocrit was 48 Despite infusions of buffered electrolyte *solutions and plasma the haematocrit rose to 55 ,* and anuria developed Suddenly the blood pressure was un ainable and metaraminol was required to keep the .olic pressure at 100 mm Hg Respiratory failure devel d There was combined metabolic and respiratory acidosis with pH 7 03 standard bicarbonate 13 8 mEq/ litre and Pco 67 0 mm Hg The electrolytes were *normal creatinine 3 1 mg/100 ml The acidosis was* corrected with sodium bicarbonate and the respiration was assisted The systolic blood pressure was kept at 100 mm Hg with metaraminol plasma and electrolyte solution during three hours in this time the extremities became warm and a urinary output of 30 ml/h was achieved after 60 ml of 20 mannitol Then the blood pressure *went down again Despite increasing amounts* of i v metaraminol and hypertensin it was impossible to keep the systolic pressure above 60 mm Hg and after a period of respiratory arrest and cardiac arrhythmia there was uncorrigible cardiac arrest

The values for catecholamines and VMA were extremely high total catecholamines were 19 00 µg/?4 h (normal below *150* µg/24 h) and VMA *398 mg/24 h (normal* below 8 mg/24 h) These results became available 8 days after admission, i e 4 days after the patient s death

At post mortem examination the heart was found to be normal in size with pale papillary muscles The lungs were moderately congested and there was slight pleural and pericardial effusion. In the oesophagus stomach and duodenum no ulcers were found *The ventricular mucosa* however was congested with multiple petecchiae The peritoneal space contained sanguinous fluid and had mul tiple fibrinous adhesions The ascending aorta had several atheromatous plaques one of which was necrotic Another necrotic plaque was found distal to the renal arteries Over the left kidney there was an encapsulated tumour the size of a grape fruit It weighed 300 g was pale greyish yellow in colour and of a soft smeary consistence

At histopathological examination (Prof C con Numers M D ) the tumour was seen to consist of malignant tissue which was *cell rich* and to a great extent necrotic The histological finding was characteristic of phaeochromocy toma The gastric mucosa showed necrosis of the super ficial part and in its deeper layers profound diffuse lymphocytic and plasmacellular inflammatory infiltration was seen The submucosa was fibrotic The findings sug *gested atrophic gastritis The skin tumours were well* dif ferentiated and consisted of fibroblasts and collagen fibres The findings were those of dermatofibroma

## DISCUSSION

*The cause of necrosis of phaeochromocytoma with* subsequent massive liberation of catecholamines is obscure In the literature concerning phaeo chromocytoma necrosis as cause of shock only one case (4) showed pure necrosis of the tumour whereas in the other cases haemorrhage into the tumour was reported (3 6 8 9 10) The possi bility that phentolamine administration caused ischaemic necrosis as suspected by Delaney and Paritzky (4) seems unlikely in our case particular ly as profound hypotension of long duration never occurred From our point of view the malignant growth in combination with the firm capsule may have produced high intracapsular pressure and subsequent necrosis

The output of catecholamines from the tumour must have been massive as judged by the cate cholamine excretion and we have *in fact not* found reports of nearly as high excretion else where The highest value reported by Sjoerdsma et al (14) was about 9000 µg/24 h in one of 64 patients with phaeochromocytoma Sack (13) re ported 2244 µg/24 h in his review of 11 patients and French (5) found about 1400 µg/24 h in a patient with phaeochromocytoma and shock The catecholamine excretion has not been reported before in patients in whom destruction of the tumour caused the acute situation (3 4 6 8 9 10) The excretion of 19 200 µg/24 h in our pa tient must be considered to be very high and was probably caused by the necrosis of the tumour If this was the case such extremely high cate cholamine excretion values may indicate the need for emergency surgery in phaeochromocytoma

*Gastric haemorrhage* associated with pheochro- mocytoma has not been reported before In our case the bleeding was diffuse The histological

changes in the gastric mucosa were obviously caused by ischaemic necrosis due to vasoconstriction following the excessive catecholamine liberation from the tumour An analogous situation was described by Novey and Meleyco (12) who observed haematemesis in a patient who was accidentally given 30 mg of epinephrine intravenously

As a clinical problem the terminal irreversible shock deserves mention In a review of shock mechanisms in phaeochromocytoma French and Campagna (5) mentioned overshooting compensatory mechanisms after withdrawal of pressor amines and a possible hypovolaemia as suggested by Brunjes et al (1) In our case we feel that irreversible shock was caused by tissue hypoxia from vasoconstriction with impaired peripheral blood flow in combination with adrenergic cardiomyopathy The impaired blood flow also caused necrosis of the gastric mucosa as well as the anuria The adrenergic myop thy seen at autopsy was suggested by the clinical findings including the electrocardiogram all of which were consistent with adrenergic cardiomyopathy as reviewed by French and Campagna (5) and Bucher et al (2)

The occurrence of phaeochromocytoma and dermatofibromatosis in the same patient should be noted Phaeochromocytoma is known to occur in combination with various neuroectodermal disorders (7 11) Dermatofibroma however is a mesodermal tumour and the simultaneous occurrence of the two tumours may well be coincidental

## REFERENCES


1 Brunjes S Johns V J & Crane M G Pheochromocytoma, postoperative shock and blood volume New Engl J Med 62 393 1960
2 Bucher H W Aepli R Zenger F & Stucki P Phaochromozytom mit aussergewohnlichen Herzrhythmusstorungen und ihre Behandlung mit alpha und beta Rezeptoren Blockern. Schw med Wschr 99 956 1969
3 Cahill F J Sympathoblast pheochromoblast. Penn med J 47 664 1964
4 Delaney J P & Paritzky A Z. Necrosis of a pheochromocytoma with shock New Engl J Med 280 1394 1969
5 French C & Campagna F A. Pheochromocytoma with shock, marked leukocytosis, and unusual electrocardiograms. Ann. intern. Med. 55 127 1961
6 Gilliland I. C & Daniel, O Phaeochromocytoma presenting as an abdominal emergency Brit. Med. J 2 275 1951
7 Glushien, A S Mansuy M M & Littman D S Pheochromocytoma its relationship to the neurocutaneous syndromes Amer J Med 14 318 1953
8 Huston J R. & Stewart, W R C Hemorrhagic pheochromocytoma with shock and abdominal pain Amer J Med. 39 502 1965
9 Jelliffe R. S Phaeochromocytoma presenting as a cardiac and abdominal catastrophe Brit. Med. J 2 76 1952
10 Lehman, D J & Rosof, J Massive hemorrhage into an adrenal pheochromocytoma New Engl J Med 254 474 1956
11 Mulholland S G Nuzhet, O A & Walzak, M P Familial pheochromocytoma associated with cerebellar hemangioblastoma J.A.M.A 207 1709 1969
12 Novey H S & Meleyco L N Alarming reaction after i v epinephrine J A M.A 207 2435 1969
13 Sack H Neuhaus J., Schega W & Korner M Die Bedeutung der medikamentosen Blockade adrenerger alpha und beta Rezeptoren fur die konservative und operative Behandlung des Phaochromocytoms Dtsch med Wschr 93 151 1968
14 Sjoerdsma, A Engelman, K. Waldmann, T A Cooperman L. H & Hammond W G Pheochromocytoma current concepts of diagnosis and treatment Ann intern Med 65 1302, 1966

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# FASTING ELECTROCARDIOGRAM

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*Ab t act* Three patients are presented with T wave in version after food intake In all cases the ECG was normal in fasting condition and after exercise Abnormal T waves are found during digitalis treatment in electro lyte disturbances, and also during anxiety Postprandial changes in the T waves have been described in rather few publications but a review of the literature seems to indicate that the changes are due to potassium move ments over the cellular membrane It is emphasized that a negative T wave in itself is not an indication of myo cardial disease In doubtful cases a fasting ECG should always be taken.

Th interpretation of the ECG is often difficult especially concerning variations in the T waves and may be impossible without a case history and clinical examination For this reason it is impor tant to emphasize that factors not directly relat ing to the heart may give rise to abnormalities in the ECG and may in this way lead to a wrong diagnosis of organic heart disease

The purpose of this article is to focus attention on the fact that food intake in some individuals may give rise to negative T waves in the extremity and precordial leads so that the postprandial ECG apparently seems abnormal with the risk of lead ing to an erroneous diagnosis of myocardial dam age As this phenomenon is not always remem bered we consider it worth while to present the case reports of three persons who had normal fasting ECG but negative T waves in the post prandial ECG At the same time a short review of the literature will be given

## CASE REPORTS

*Pat e t 1*

The patient was a 28 year-old male who had prev ously been free from heart symptoms He had never had rheumatic fever nd there was no family history of heart disease From 1959 to 1961 he had been in military service which he had well tolerated

In 1967 he was work ng in Greenland as a mechanic After two months he suddenly became ill with anxiety spells and hyperventilation symptoms He was admitted to a local hospital and a heart infarction was suspected because of negative T waves in the lateral precordial leads There was no fever no leucocytosis and the serum enzymes were normal He was transferred to our depart ment

On admission he still had spells of anxiety

The examination showed nothing abnormal There was no cyanosis or dyspnea.

Auscultation of the heart was normal and no mur murs were detected.

Blood pressure was 130/80 mm Hg.

Hb 15 0 g% ESR was 3 mm in one hour Leucocytes 3600 per $\mu$l

LDH 10 units (normal) G P transaminase 1 3 unit per ml (normal)

Se-cholesterol 211 mg %

Roentgenogram of the chest showed a normal heart

Electrocardiogram (Fig. 1) showed low T waves in leads I and II and negative or isoelectric T waves in the precordial leads Fasting ECG (Fig. 2) showed posi tive T waves in leads I and II and in $V_{4-5-6}$ ECG during exercise (Fig. 3) was normal

The patient was seen one year later and the ECG showed the same T wave changes before and after meals

Conclusion no sign of organic heart d sease

*Patient 2*

A 21 year-old woman who was seen in the department during her first pregnancy because of a systolic murmur

She had never had rheumatic fever nor heart symptoms During the pregnancy she was feeling completely well

At auscultat on of the heart a faint systolic murmur of vibratory type was heard The second sound in the pulmonary area was normally split There was no sign of cardiac incompensation Blood pressure 150/80 mm Hg

No roentgenogram of the chest was taken because of the pregn ncy The ECG (Fig 4) showed isoelectric T waves in leads I and II negative T waves in V ... and isoelectric in $V_6$ Fasting ECG (F g. 5) completely normal with normal T waves in all leads.

The deli ery was uneventful and the patient was seen one year after deli ery She still had no heart symptoms

The ECG was unchanged, with negati e T waves in postprandial condition Fasting ECG was still normal

Roentgenogram of the chest was normal

Conclus on no o ganic heart disease

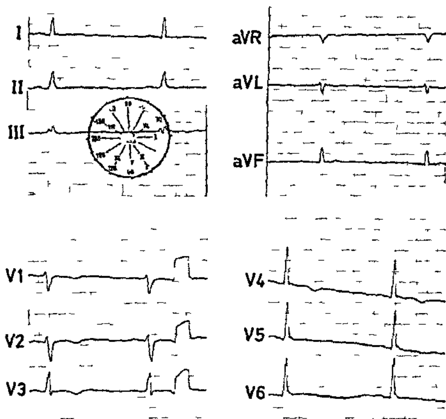



Fig 1 Patient 1 Postprandial ECG

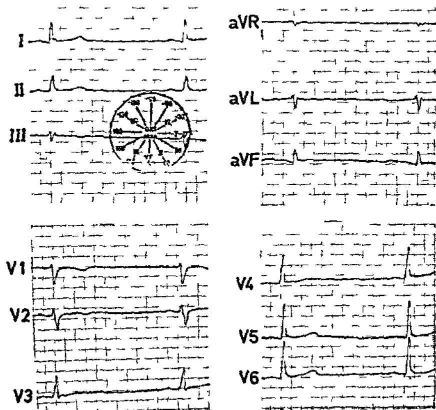



Fig 2 Patient 1 Fasting ECG

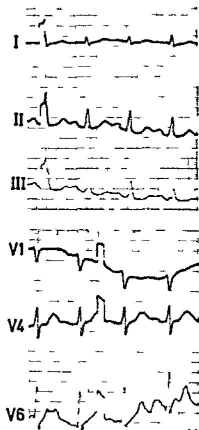


*Fig 3* Patient 1 Exercise ECG

*Patient 3*

A 33 year-old male who was admitted to the department because of attacks of dizziness

He had never had rheumatic fever and there was no family history of heart diseases. Up to the age of twenty he had had frequent attacks of otitis, but otherwise had been completely healthy

During the last two years before admission to the department he had had attacks in which he felt shortness of breath, accompanied by symptoms of hyperventilation On two occasions he had short lasting black-outs but had never fainted. During these attacks he had no chest pains or feeling of tachycardia. At the objective examination no dyspnea or cyanosis was found.

Auscultation of the heart was normal Blood pressure was 130/80 mm Hg in supine position 115/90 standing

Roentgenogram of the chest was normal Heart volume 325 ml/m

The EEG was normal

The ECG (Fig. 6) showed diphasic negative T waves in $V_{3-4}$

Fasting ECG (Fig 7) showed normal positive T waves in all leads.

ECG during exercise was normal

Conclusion no organic heart disease

## DISCUSSION

Changes in the T waves in the ECG are seen in several conditions not due to myocardial disease In the first place these changes may be seen during digitalis treatment and in diseases with electro-

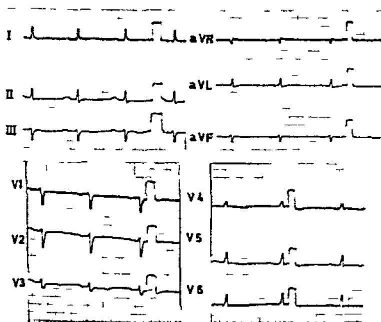


*Fig 4* Patient 2 Postprandial ECG

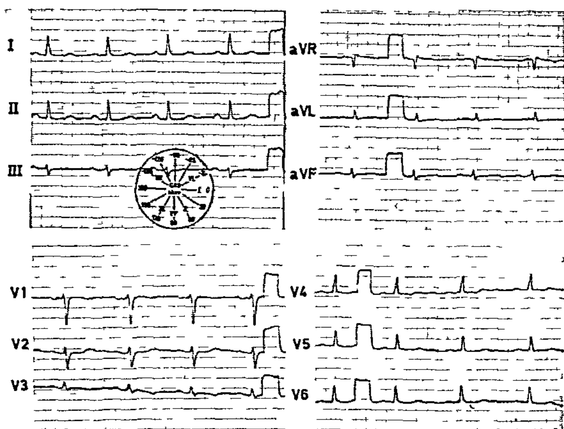



Fig 5 Patient 2 Fasting ECG

lyte disturbances As withdrawal of digitalis or correction of the abnormal electrolyte concentration most often results in a normal ECG the *interpretation of the ECG in such cases is not* difficult.

Physiological negative T waves are found in young negro males (3) More difficult to appraise are the changes in the T waves seen during anxiety Mainzer and Krause (6 7) in 1939 and 1940 reported series of patients without heart disease in whom an ECG was taken before operation and before any anesthetic or preanesthetic drug was given In 30 and 53 cases changes in the T waves and ST-segments were seen After the operation the ECG was again normal No description has ever been given of the effect fasting would have in patients with anxiety-caused T wave changes Patient 1 and possibly patient 3 in this study had anxiety so that the two phenomena may be found in combination

Temperature has some influence upon the T waves in animals In 1923 Smith (10) described how cooling of the myocardium of dogs produced inversion of the T waves How cold would have *an influence on the human myocardium has not* been reported

The effect of food intake on the ECG has been described. In 1939 Gardberg and Olsen (1) reported a series of nine young healthy men of whom seven had negative T waves after an ordinary meal The ECG was normalized 2 to 3 hour after the meal In 1946 Simonsen et al (9) examined 12 normal men (all were volunteers assigned to his laboratory for Civilian Public Service by the Selective Service System) The age of the men was between 19 and 32 years. ECGs were taken before and 30 to 60 min after a meal either ordinary or fatty meal Negative T waves were recorded in the precordial leads in all cases, irrespective of whether they had had an ordinary or fatty meal.

Hiss and Lamb (4) found inverted T waves in

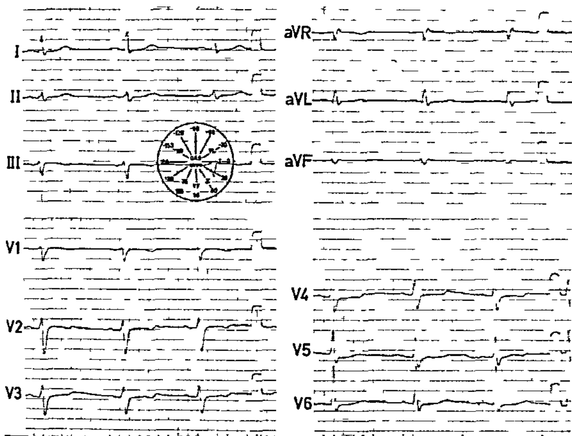



*Fig 6* Patient 3 Postprandial ECG

32 persons in a series of 122 043 subjects ranging from 16 to 50 years of age all healthy men Sixty five per cent of these persons had a normal ECG in fasting state mainly men under forty

Klepzig and Tecklenborg (5) reported in 1957 that in 85 healthy persons 55 had T wave changes after a meal In these cases the changes were not related to heart rate or to serum potassium Nor were the changes related to posture or to disloca tion of the heart due to filling of the stomach as glucose given intravenously produced the same changes

Rochling and Edwards (8) in 1954 reported four cases in whom 100 g glucose produced negative T waves in the lateral precordial leads

It is interesting to note that in these cases insulin caused the same changes and that potas sium could prevent the changes completely The same authors reported the effect of glucose in take in 85 normal persons of whom 55 had changes in the ECG

Goldman in 1960 (2) presented a review of this problem which he called the isolated T negativity syndrome" He found that fasting nor malized the ECG as also did exe cise in all cases He reported five patients who were misinterp eted solely because of the n gative T waves One of these had become a heart invalid and one patient underwent thoracotomy on suspicion of pericar dial disease but nothing abnormal was found All these five patients had normal ECG in the fasting condition.

The explanation of the changes in the ECG after meals is not clear but the reported experi ments with insulin and potassium strongly indi cate that shifting of potassium over the cellular membranes possibly after release of insulin could be the explanation of the phenomenon

Table I. *Sequence of administration of diuretic treatments*

| No. of pat. | Days 1 | 2 | 3 |
|---|---|---|---|
| 2 | A | B | C |
| 2 | A | C | B |
| 2 | B | A | C |
| 2 | B | C | A |
| 2 | C | A | B |
| 2 | C | B | A |

*Treatments:* A, ethacrynic acid; B, mercaptomerin; C, furosemide.

chloride and 40 mEq potassium daily. The fluid intake was *fixed at 1500 ml per day* in programme I and at 2000 ml daily in programme II.

The urine was collected in twenty-four-hour periods and urinary excretion of water, osmolal clearance, free water clearance, sodium, potassium, chloride and net acid was determined according to methods previously described (17). The serum osmolality, sodium, chloride, standard bicarbonate and creatinine were determined every morning in the fasting state from the 4th to the 7th day. Body weight was measured every morning.

The administration of diuretics which started on the *4th day in the morning* followed the scheme shown in Table I, where A indicates ethacrynic acid, B mercaptomerin and C furosemide. Through this scheme all diuretics had an equal chance of showing their effects regardless of the variations caused by different patients and by varying sequence of administration of drugs. A random allocation of treatment programmes to patients was secured.

The study was divided into two parts: a low dose programme (I) and a high dose programme (II). Each *programme included* 12 patients. In programme I the dose levels were: ethacrynic acid (Edecrin®) 100 mg orally, mercaptomerin (Thiomerin®) 125 mg (= 1 ml) intramuscularly and furosemide (Lasix®) 80 mg orally.

In programme II double doses were given: ethacrynic acid (Edecrin®) 200 mg orally, mercaptomerin (Thiomerin®) 250 mg (= 2 ml) intramuscularly and furosemide (Lasix®) 160 mg orally. The oral diuretics were all given in divided doses at 8 a.m. and at 1 p.m. In programme II 3 g of potassium chloride were given daily in order to prevent marked serum electrolyte disturbances.

The statistical analysis takes advantage of the design of the study which allows account to be taken of the variations due to different patients and to different sequence of administration of drugs. Through analysis of variance the error variance is reduced and the significance of the effects of the treatments alone is increased. The effects of treatments may be resolved in two comparisons by the use of the rules of orthogonality. The first comparison is between two groups of treatments, and the second comparison is between the sum of these treatments and the third treatment (10, 17, 22).

It is a prerequisite for the design of the study that the action of diuretics has ceased before the administration of the next treatment group. Available reports appear to indicate that this holds true for ethacrynic acid (2, 7, 8), mercaptomerin (6, 12) and furosemide (4, 17, 23).

## RESULTS

### I. *Weight Loss and Diuresis*

The mean 24-hour values for weight loss and for renal water and electrolyte excretion after each type of treatment are given with statistical analysis in Table II. It appears from this table that a large part of the variations in response is caused by different patients and by differences in days of treatment. In the following analysis the significance of the effects of treatments alone is examined after elimination of the effects of the variables mentioned above. In programme I the effect of furosemide (C) (80 mg orally) appeared to be superior to that of ethacrynic acid (A) (100 mg orally) and of mercaptomerin (B) (1 ml intramuscularly). The mean weight loss was significantly higher after furosemide than after ethacrynic acid and mercaptomerin, as shown by the comparisons A–C and B–C in Table I. Similarly the diuresis was significantly higher after furosemide than after mercaptomerin ($p < 0.01$), while the comparison between furosemide and ethacrynic acid showed the same trend. Statistically significant differences between the effects of ethacrynic acid and mercaptomerin did not appear, although the mean weight loss and mean diuresis were higher after the former drug.

In programme II the effects of furosemide (160 mg) and of ethacrynic acid (200 mg orally) were definitely superior to that of mercaptomerin (2 ml intramuscularly). The comparisons A–B and B–C bear this out for weight loss and for diuresis, and the ortogonal comparison $A + C - 2B$ confirms the fact that the effect of mercaptomerin is inferior to that of the two oral drugs. While the mean values for weight loss and diuresis were higher after furosemide than after ethacrynic acid, a statistically significant difference was not found.

### II. *Sodium, Potassium, Chloride and Net Acid Balance*

#### (a) *Urinary excretion*

The mean values for sodium excretion showed the same trends as found for weight loss and diuresis.

Table II *Statistical analysis of body weight changes and urinary water and electrolyte excretion*

| | | Mean 24 h values for each treatment | | | Variance ratios | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Sources of variation | | Comparisons | | | | | |
| | Units | A | B | C | Patients | Days | Treatments | (A − B) | (A + B − 2C) | (A C) | (A + C−2B) | (B C) |
| Significance | F99 | | | | 3 29 | 5 85 | 5 85 | 8 10 | 8 10 | 8 10 | 8 10 | 8 10 |
| Limits | F95 | | | | 2 31 | 3 49 | 3 49 | 4 35 | 4 35 | 4 35 | 4 35 | 4 35 |
| *Prog amme I* | | | | | | | | | | | | |
| Body weight | kg/24 h | −0 36 | −0 12 | −0 82 | 0 77 | 3 14 | 5 59 | 1 29 | 9 92 * | 4 66 | 6 55 | 10 88 * |
| *Urinary excretion* | | | | | | | | | | | | |
| Diuresis | ml/24 h | 1362 | 1119 | 1634 | 1 31 | 3 79 | 6 61 | 2 94 | 10 28 | 3 68 | 9 54 | 13 21 * |
| Na + K | mEq/24 h | 130 | 125 | 177 | 2 13 | 2 47 | 4 01* | 0 09 | 7 93 | 5 22 | 2 79 | 6 71 |
| Na | mEq/24 h | 68 | 74 | 108 | 3 42 | 3 36 | 3 43 | 0 14 | 6 72 | 5 94 | 0 92 | 4 21 |
| K | mEq/24 h | 62 | 51 | 69 | 1 03 | 0 48 | 2 83 | 2 06 | 3 60 | 0 86 | 4 80 | 5 58 |
| Net acid | mEq/24 h | 72 | 57 | 79 | 1 86 | 0 04 | 1 43 | 1 17 | 1 70 | 0 34 | 2 53 | 2 79 |
| Cl | MEq/24 h | 83 | 82 | 121 | 2 89 | 3 67* | 2 58 | 0 00 | 5 16 | 3 79 | 1 37 | 3 96 |
| Free water clearance | ml/24 h | −731 | −944 | −947 | 2 47* | 1 26 | 2 14 | 3 16 | 1 12 | 3 26 | 1 02 | 0 00 |
| Osmolal clearance | ml/24 h | 2093 | 2063 | 2582 | 1 03 | 3 15 | 4 72 | 0 02 | 9 42 | 6 65* | 2 79 | 7 50 |
| Creatinine | mg/24 h | 1415 | 1213 | 1470 | 3 08 | 0 14 | 1 05 | 1 13 | 0 96 | 0 10 | 2 00 | 1 91 |
| *Programme II* | | | | | | | | | | | | |
| Body weight | kg/24 h | −0 65 | +0 05 | −0 89 | 3 39 * | 0 41 | 4 66 | 4 88* | 4 44* | 0 50 | 8 82** | 8 58* |
| *Urinary excretion* | | | | | | | | | | | | |
| Diuresis | ml/24 h | 2295 | 1551 | 2451 | 2 68* | 1 10 | 4 02 | 5 15 | 2 88 | 0 11 | 7 93* | 6 80 |
| Na + K+ | mEq/24 h | 256 | 173 | 281 | 7 42* | 0 91 | 5 19 | 5 59 | 4 80 | 0 51 | 9 88 | 9 48 |
| Na | mEq/24 h | 153 | 103 | 188 | 10 36 | 1 06 | 4 35 | 3 01 | 5 69 | 1 43 | 7 28* | 8 62* |
| K+ | mEq/24 h | 103 | 70 | 93 | 2 82* | 0 55 | 5 88 | 11 16 | 0 61 | 0 98 | 10 79 | 5 52 |
| Net acid | mEq/24 h | 51 | 43 | 38 | 1 75 | 1 41 | 1 95 | 1 30 | 2 60 | 3 87 | 0 03 | 1 74 |
| Cl | mEq/24 h | 219 | 114 | 235 | 4 42 | 0 85 | 5 63 | 7 12 | 4 15 | 0 18 | 11 09 | 9 60 |
| Free water clearance | ml/24 h | −511 | −750 | −557 | 5 63 * | 0 49 | 1 15 | 2 04 | 0 26 | 0 07 | 2 23 | 1 34 |
| Osmolal clearance | ml/24 h | 2806 | 2301 | 3008 | 3 78 | 1 21 | 1 46 | 1 41 | 1 52 | 0 22 | 2 70 | 2 76 |
| Creatinine | mg/24 h | 973 | 874 | 853 | 5 73 | 1 21 | 1 01 | 1 19 | 0 83 | 1 79 | 0 24 | 0 05 |

*Abbr ıı ı ons*
Treatments A ethacrynic acid B mer aptomerin C furosemid
Code for statistical signifi ance *P* l ss than 0 05 *P* l ss than 0 01

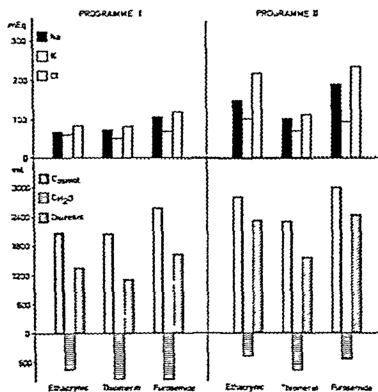



*Fig. 1.* Mean 24-hour values for renal electrolyte excretion, diuresis, osmolal and free water clearance after each type of diuretic treatment.

le II and Fig. 1). In programme I the sodium was higher after furosemide than after the two other drugs. The comparison A–C was significant at the 5% level while the comparison B–C did not reach the significance level. In programme II the mean sodium outputs were higher after furosemide and ethacrynic acid than after mercaptomerin. The comparison B–C was statistically significant at the 1 percent level.

The pattern of potassium excretion was different from that of sodium (Table II and Fig. 1).

In both programmes the mean values for K output were lower after mercaptomerin than after both other drugs. In programme I the comparison B–C and in programme II the comparisons A–B and B–C were statistically significant. The finding that the orthogonal comparisons $A+C-2B$ were statistically significant in both programmes stresses the fact that the potassium excretion was lower after mercaptomerin than after both other drugs.

According to current views the urinary potas-

Table III. *Potassium excretion in relation to sodium output*

| | Natriuresis below 150 mEq/24 h | | | Natriuresis above 150 mEq/24 h | | |
|---|---|---|---|---|---|---|
| | No. of pats. | Mean Na output (mEq) | Mean K output (mEq) | No. of pats. | Mean Na output (mEq) | Mean K output (mEq) |
| *Programme I* | | | | | | |
| Ethacrynic acid | 11 | 57 | 61 | 1 | 198 | 73 |
| Mercaptomerin | 10 | 53 | 48 | 2 | 183 | 53 |
| Furosemide | 9 | 88 | 68 | 3 | 168 | 72 |
| *Programme II* | | | | | | |
| Ethacrynic acid | 7 | 73 | 97 | 5 | 165 | 110 |
| Mercaptomerin | 9 | 48 | 71 | 3 | 167 | 74 |
| Furosemide | 6 | 83 | 88 | 6 | 293 | 99 |

sium excretion takes place in the distal segment of the nephron where potassium is secreted in exchange with sodium which is reabsorbed from the tubular fluid This process is dependent upon the tubular supply of sodium and the activation of the exchange mechanisms (1 16) In the present study the low potassium output after mercaptomerin is associated with a low sodium excretion and it might therefore be explained by a low supply of sodium to the exchange sites In order to evaluate the significance of this factor the potassium excretion was examined in relation to the level of sodium output for each treatment as shown in Table III It appears that the K excretion is persistently lower after mercaptomerin than after both other drugs also with high sodium excretion values This finding suggests strongly that there is a significant difference in the rate of sodium potassium exchange in the distal renal tubules after mercaptomerin as compared to furosemide and ethacrynic acid

It follows from the differences shown in the rate of sodium potassium exchange after different diuretics that an analysis of the cation excretion i e the Na+K output would be more relevant as an index of saluretic action than a study of

Table IV *Alterations in serum electrolytes in relation to diuretics*

| Parameter programme | Typ of diuretic treatment | | |
|---|---|---|---|
| | Ethacrynic acid | Mercaptomerin | Furosemide |
| Serum Na (mEq/l) | | | |
| I Mean | +0 6 | −0 4 | +1 0 |
| Range | −5–+2 | −3–+1 | −5–+4 |
| II Mean | +1 0 | +0 3 | +0 4 |
| Range | −3–+7 | −4–+6 | −2–+3 |
| Serum Cl (mEq/l) | | | |
| I Mean | −2 1 | −0 6 | −1 3 |
| Range | −6–0 | −4–+2 | −3–+3 |
| II Mean | −0 6 | +0 3 | −0 8 |
| Range | −3–+3 | −3–+3 | −10–+2 |
| Serum K (mEq/l) | | | |
| I Mean | −0 18 | −0 06 | −0 12 |
| Range | −0 8–+0 | −0 6–+0 6 | −0 9–−0 6 |
| II Mean | −0 6 | +0 07 | −0 22 |
| Range | −0 9–+0 5 | −0 6–+0 6 | −0 6–+0 2 |
| Stand HCO (mEq l) | | | |
| I Mean | +0 57 | +0 19 | +1 33 |
| Range | −2 3–+2 3 | −3 3–+2 4 | −1 0–+3 0 |
| II Mean | +0 51 | −0 13 | +0 93 |
| Range | −2 2–+4 0 | −2 5–+2 2 | −3 2–+4 3 |

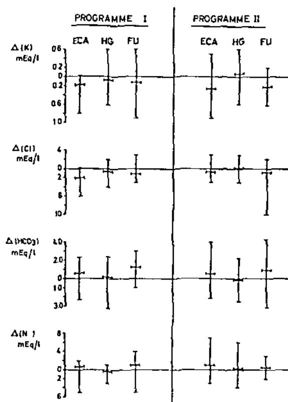



*Fig 2* Mean values and ranges of changes in serum electrolyte concentrations after each type of diuretic treatment

the single ions Actually the total cation excretion (Na+K) followed closely the pattern of the weight loss as shown by the statistically significant differences in comparisons A–C and B–C in programme I and by the comparisons A–B and B–C in programme II Accordingly the analysis of the cation excretion confirms that furosemide is more potent than mercaptomerin and ethacrynic acid in programme I and that furosemide and ethacrynic acid have a greater effect than mercaptomerin in programme II

The pattern of chloride excretion followed that of the cations as shown in Table II and Fig 1 In programme I the comparisons A+C−2B was significant and in programme II the comparisons A–B and B–C reached the significant levels The net acid excretion did not show any significant trends

In both programmes the total creatinine excretion values were apparently unaffected by the diuretic treatments

Table V *Serum electrolytes in relation to diuretic treatment*

| | Control value before trial | Values before each diuretic treatment | | |
|---|---|---|---|---|
| | | Ethacrynic acid | Mercaptomerin | Furosemide |
| *Programme I* | | | | |
| Serum Na (mEq/l) | | | | |
| Mean value | 139 0 | | | |
| Range | 135–142 | | | |
| Serum Cl (mEq/l) | | | | |
| Mean value | 101 5 | 100 6 | 100 2 | 100 4 |
| Range | 96–106 | 95–104 | 95–106 | 95–106 |
| *Serum stand –$HCO_3$ (mEq/l)* | | | | |
| Mean value | 24 04 | 24 79 | 24 69 | 24 91 |
| Range | 20 3–28 1 | 21 8–29 0 | 21 9–31 1 | 20 3–27 3 |
| *Programme II* | | | | |
| Serum Na (mEq/l) | | | | |
| Mean value | 135 5 | | | |
| Range | 129–143 | | | |
| Serum Cl (mEq/l) | | | | |
| Mean value | 97 4 | 97 0 | 96 4 | 97 8 |
| Range | 87–104 | 87–103 | 84–101 | 84–104 |
| Serum stand $HCO_3$ (mEq/l) | | | | |
| Mean value | 24 63 | 25 47 | 25 61 | 25 27 |
| Range | 19 8–28 1 | 2[illegible] 0–28 1 | 19 8–29 9 | 22 0–33 1 |

(*b*) *Serum electrolytes*

mean values and ranges for variation of .im electrolytes after each type of diuretic eatment are shown in Table IV and in Fig 2 Serum sodium level was essentially unchanged after mercaptomerin but showed a trend to rise after ethacrynic acid and furosemide

A tendency to a decrease of serum chloride is noted in particular after ethacrynic acid and furo semide Correspondingly there is a trend to in crease of serum standard bicarbonate after these drugs The range of changes is very similar after all three drugs

In accordance with the tendency to a smaller K output after mercaptomerin than after the other drugs the mean values for serum potassium changed little during treatment with this drug A decrease of mean serum potassium is seen after ethacrynic acid and furosemide As found above however the range of changes is very similar after all three drugs and serum potassium values below 3 5 mEq/l were seen after mercaptomerin in four patients in programme I and in two patients in programme II

Serum creatinine levels were unchanged during the trial

III *Free Water Clearance*

While the mean values for osmolal clearance tend ed to follow the pattern of diuresis and of weight loss the mean values for the negative free water clearance showed a different pattern (Table II and Fig 1)

In programme I the negative free water clear ance i e the tubular reab orption of solute free water had a lower mean value after ethacrynic acid than after mercaptomerin and furosemide In programme II the tubular reabsorption of sol ute free water was lower after ethacrynic acid and furosemide than after mercaptomerin Al though these trends are not of statistical signifi cance they suggest a difference in the renal tubular reabsorption of solute free water between the three diuretics The trends indicate that ethacrynic acid and furosemide in a larger dose produce a urine with a lower solute water ratio than mer captomerin This results in a relatively higher water output and leaves the body with a higher total solute total water ratio However although the serum sodium levels tended to rise a little more after ethacrynic acid and furosemide than after mercaptomerin the trends were not signifi cant

VI *Diuretic response in relation to acid base balance*

| | Serum standard bicarbonate | | | | | |
|---|---|---|---|---|---|---|
| | No of pats | | Diuresis (ml/24 h) | | Na + K excretion (mEq/24 h) | |
| | (<25 0 mEq/l) | (≥25 0 mEq/l) | (<25 0 mEq l) | ((≥25 0 mEq l) | (<25 0 mEq l) | (≥25 0 mEq/l) |
| rynic acid | 12 | 12 | 1684 | 2046 | 169 | 219 |
| ptomerin | 10 | 14 | 1663 | 1100 | 178 | 130 |
| emide | 11 | 13 | 1988 | 2086 | 215 | 227 |

*ffects of Diuretics in Relation to Acid Base Balance*

well established that mercurial diuretics have creased diuretic action in the presence of hloraemia and metabolic alkalosis (6 11 15 20) and there is good evidence that acid disturbances are more important in this xt than the serum chloride levels (13) It is nent therefore to analyze the significance e acid base balance for the diuretic response e present study

ble V shows the mean values and ranges of sodium chloride and serum standard bi onate before each treatment It will be noted the patients in programme II present a moder ecrease of the mean serum sodium level and equently of the average serum chloride con ration as compared to the patients in pro- e I However the standard bicarbonate n values are nearly equal and approximately of the patients in each group have standard rbonate values above 25 0 mEq/l (normal e 19 0–24 5 mEq/l)

shown in Table VI the combined data of programmes demonstrate clearly that the t of mercaptomerin in terms of diuresis and n excretion is decreased when serum standard rbonate is elevated above 25 mEq/l whereas action of ethacrynic acid and furosemide is anged

## DISCUSSION

planning and statistical analysis used in this y permit an unbiased comparative evaluation the effects of the diuretics mercaptomerin, crynic acid and furosemide separate from the cts of different patients and of varying se nce of administration of drugs (10 17)

is apparent from the analysis of weight loss diuresis and cation excretion that furosemide (80 mg) has a greater effect than mercaptomerin (125 mg (= 1 ml) intramuscularly) and ethacrynic acid (100 mg orally) and that furosemide (160 mg) and ethacrynic acid (200 mg orally) are superior to mercaptomerin (250 mg (= 2 ml) intramuscularly) These results are in agreement with several other reports in which mercurial diuretics have been compared either with ethacrynic acid (2 12 21 25) or with furosemide (23 25) even though the significance of different patients and of vary ing sequence of administration of drugs has not been taken into account

Two points are of particular interest in the discussion of the comparative efficacy of the three diuretics 1) the dose levels compared and 2) the significance of acid base balance for the diuretic response

In terms of dose levels we have not exceeded the usually accepted maximal dose of 2 ml of mercaptomerin and have compared it to furo- semide (160 mg) and ethacrynic acid (200 mg) In patients with refractory heart failure Siegel and Gifford (21) compared the effects of the mercurial diuretic meralluride (4–6 ml) with ethacrynic acid (100–450 mg) and found that ethacrynic acid on the average was the superior diuretic Since the accepted maximal dose of meralluride is 2 ml it appears unlikely that the use of higher doses of mercaptomerin in the present study would have changed the above conclusions

The significance of acid base status before administration of the diuretics is clearly borne out by the present study which confirmed that meta bolic alkalosis has an inhibitory effect upon the diuretic response to mercaptomerin (13) whereas this effect was not found in the case of ethacrynic acid or furosemide Apparently the presence of slight metabolic alkalosis in about half o

patients is able to account for at least a part of the difference in diuretic response between mercaptomerin and the two other drugs, and the effects of a pretreatment with acidifying agents upon the response to mercaptomerin remain to be examined. Consequently the superiority of large doses of furosemide and of ethacrynic acid as compared to mercaptomerin holds true only for patients who have previously received diuretics and may present some degree of metabolic alkalosis as is often found in advanced heart disease.

With regard to the solute-water relationship during diuresis, studies in man have indicated differences in the type of action between ethacrynic acid and furosemide on the one hand and mercaptomerin on the other. While the former drugs inhibit the renal tubular reabsorption in the ascending limb of Henle's loop and decrease the hypertonicity in the renal medulla, causing a reduction of the renal concentrating capacity (5, 7, 16, 23, 24, 25), the mercurials do not present this action (6, 9, 27). A manifestation of this difference in type of action between the water diuretics during clinical trials would be of importance when in normal man and in patients with congestive heart failure on limited water supply excreted urine is hypertonic and water balance maintained through a tubular reabsorption of solute-free water. A significant decrease of renal concentrating capacity would result in a limitation of tubular reabsorption of solute-free water during diuresis and an increased output of water in relation to total solute excretion. As a result the body would be left with a higher solute-water ratio than before.

In the present study the tubular reabsorption of solute-free water was lower after high doses of ethacrynic acid and furosemide than after mercaptomerin. In accordance with these findings the serum sodium levels tended to rise a little more after ethacrynic acid and furosemide than after mercaptomerin. These trends are not statistically significant although they appear to be in agreement with the established data on the pharmacological effects of the diuretics. However, as a natural corollary these trends would appear to suggest that ethacrynic acid and furosemide might be more useful in the correction of dilutional hyponatremia than the mercurial diuretics (14–21).

With regard to potassium balance during diuretic treatment our results indicate that the potassium output was consistently lower after mercaptomerin than after the other two drugs. It is generally accepted that potassium excretion is governed by the rate of sodium-potassium exchange in the distal segment of the nephron and is dependent upon the supply of sodium to the exchange sites and upon the activation of the exchange mechanism. Since the sodium output was lower after mercaptomerin than after the other drugs, the lower potassium excretion after mercaptomerin might be due to a decreased availability of sodium at the exchange sites. As shown in Table III, a trend to a rise in potassium excretion with increasing sodium output is present after all three drugs but the potassium output is consistently lower after mercaptomerin than after the other two drugs. This finding is in accordance with the concept that the mercurial diuretics have a dual effect upon the sodium-potassium exchange mechanism: 1) a stimulating effect due to an increased supply of sodium, 2) an inhibitory effect upon the sodium-potassium exchange rates (1, 16).

This specific action of mercaptomerin would appear to be beneficial in terms of maintenance of total potassium balance. In accordance with this result the mean level of serum potassium changed very little after mercaptomerin, while a decrease of the mean potassium concentration was present after furosemide and ethacrynic acid. On the other hand, the range of changes of serum potassium after mercaptomerin was equal to that after the two other drugs, and marked hypokalemia was found [illegible] after mercaptomerin. Consequently, from the clinical point of view this difference in type of action does not compensate for careful observation during treatment with mercurial diuretics.

A comparison of Tables II and III reveals that the average decrements of serum chloride and the elevated increments of serum standard bicarbonate largely followed the magnitude of chloride excretion and weight loss. Therefore the small changes of mean serum chloride and bicarbonate levels observed after mercaptomerin are most likely a reflection of the smaller quantitative effect of this drug.

Ethacrynic acid and furosemide were very similar in terms of qualitative and quantitative effects at high dose levels. When compared on a

gramme basis furosemide appeared to be v more potent than ethacrynic acid
conclusion this permutation trial test has *that in patients with congestive heart fail* furosemide and ethacrynic acid are more diuretics than mercaptomerin when this is used without acidifying adjuvants The of hypokalaemia hypochloraemia metabolic alkalosis was a risk after all three Although mercaptomerin caused less potas excretion than the oral drugs and although rynic acid and furosemide tended to decrease tubular reabsorption of solute free water qualitative differences were not of such ficance as to influence severely the choice of ic.

## REFERENCES


iner R. W Renal secretion of potassium and ydrogen ions Fed Proc 11 695 1952
, A N Onesti G Seller R. Ramirez, O ider C & Moyer J H Pharmacodynamic effects f a new diuretic drug ethacrynic acid. Amer J diol 16 99 1965
A. N Seller R. Onesti G Ramirez, O artz C & Moyer J H Clinical selection of iretic drugs in the management of cardiac edema r J Cardiol 22 168 1969
st, A. N Seller R Ramirez, O Onesti G & loyer J H Comparative diuretic efficacy of furo mide J New Drugs 5 3 9 1965
ichborn E & Anastasakis, S Angriff punkt und rkungsmechanismus von Furosemid am distalen lephron des Menschen Klin Wschr 42 1127 1964
uny E The site and mechanism of action of rcurial diuretics Pharmacol Rev 20 89 1969
on P J Heinemann H O Stason W B & agh J H Ethacrynic acid Effectiveness and of action in man Circulation 31 5 1965
Goldberg M McCardy D K Foltz, E L & uemle L W Effects of ethacrynic acid (a new etic agent) on renal diluting and concentrating hanisms evidence of site of action in the loop of e J clin Invest 43 201 1964
rossman, J Weston R. E Borun, E R. & Leiter Factors influen ing the course of mercurial diu during pitressin infus on in normal subjects J *Invest 34 1611 1955*
F Permutation trial of diuretics Chlorothiazide hydroflumethiazide Circulation 21 717 1960
R. P Schoenfeld M R. & Friedberg, C K -lys ne monohydrochloride a clinical study of its tion as a chloruretic acidifying adju ant to mer rial diuretics New Engl J Med 263 728 1960
M Caranesos G J Mulholland J H & The Osler Medical House Staff Controlled study compar ng ethacrynic acid to mercaptomerin in the treatment of acute pulmonary edema New Engl J Med 279 115 1968
13 Leony R. L. Weiner I. M & Mudge G H The effects of acid base balance on the diuresis produced by *organic and inorganic mercurials J clin Invest* 37 1016 1958
14 Luckey E H & Rubin A L The correction of hyponatremia in congestive heart failure Circulation 21 229 1960
15 Mizgala H F Lasser R P & Friedberg, C K The treatment of refractory retention of fluid oral L arginine monohydrochloride and meralluride Amer Heart J 65 5 1963
16 Mudge G H Ames, A Foulks J & Gilman A. Effect of drugs on renal secretion of potassium in the dog Amer J Physiol. 161 151 1950
17 Olesen K H Dupont B & Flensted Jensen E *The combined diuretic action of quinethazone* and furosemide in congestive heart failure Acta med scand 187 33 1970
18 Puschett J B & Goldberg, M The acute effects of furosemide on acid and electrolyte excretion in man J Lab clin. Med. 71 666 1968
19 Rubin A L & Braveman, W S Treatment of the low salt syndrome in congestive heart failure by the controlled use of mercurial diuretics Circulation 13 655 1956
20 Rubin A L Spritz, N Mead A. Herrmann R A. Braveman W S & Luckey E H The use of L-lysine monohydrochloride in combination with mer curial diuretics in the treatment of refractory fluid retention Circulation 21 332 1960
21 Siegel W & Gifford, R. W Efficacy of ethacrynic acid in patients with refractory congestive heart fail ure resistant to meralluride Amer J Cardiol 2? ?60 1968
2 Snedecor G W Statistical methods Iowa State medical college Ames Iowa 1956
23 Stason W B Cannon P J Heinemann H O & Laragh J H Furosemide a clinical evaluation of its diuretic action. Circulation 34 910 1966
24 Stein J H Wilson C B & Kirkendall W M Differences in the acute effects of furosemide and etha rynic acid in man J Lab clin Med 71 654 1968
25 Stewart J H & Edwards K D G Clinical comparison of furosemide with bendrofluaz de mersa lyl and ethacrynic acid Brit med J 2 1277 1965
6 Suki W Rector F D & Seldin, D W The site of action of furosemide and other sulfonamide diu retics in the dog J clin Invest 44 1458 1965
27 Welt L G Goodyer A V Darragh J H Abele W A & *Maroney W H Site of saluretic action of* an organ c mercurial compound J appl Physiol 6 134 1953

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# PLASMA RENIN ACTIVITY AND ALDOSTERONE SECRETION RATE IN HYPERTENSION

*The Distinction between Primary and Secondary Hyperaldosteronism*

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stract. Aldosterone secretion rate (ASR) and plasma n activity (PRA) on normal sodium and potassium kes have been measured simultaneously in 14 normal ects, 14 non-malignant essential hypertensive patients, e malignant essential hypertensive patients, eight renoular hypertensive patients, two patients with renal ertensive patients and five patients with primary eraldosteronism. The effect of salt loading was ingated in the two latter groups. In all groups of ertensive patients the frequency of increased ASR was h. Fifty per cent of essential non-malignant hyperve patients had an ASR above normal range. There s no correlation between ASR and the grade of eye dus changes in essential hypertensive patients. Exing patients with primary hyperaldosteronism a pronced linear correlation between PRA and ASR was nd. This indicated that the increased ASR in these ups of hypertensive patients is secondary to renin mulation. Primary hyperaldosteronism was diagnosed by RA ASR ratio differing significantly from the normal relation. Failure of salt loading to depress ASR was t specific for primary hyperaldosteronism. In only two five patients with abnormal PRA ASR ratio was a itary adenoma found. Removal of the tumour cured of them. In the other three patients the adrenals owed adenomatous hyperplasia, and unilateral adrenalomy had no effect. It is concluded that the best erion for primary hyperaldosteronism at present is a viation of the PRA ASR ratio from the normal correlan, but differentiation between classical aldosteronoma d adenomatous hyperplasia of the adrenal gland is not yet possible.

e demonstration of the existence of cases of ormokalaemic primary hyperaldosteronism (11 ?) started the quest for methods to screen the pertensive population for this condition. Conn et suggested as screening procedure the measurement of plasma renin activity (PRA) which had een found subnormal or undetectable in classical primary hyperaldosteronism (8 10) The response of PRA to the stimuli of salt depletion and change in posture was also found impaired (10). Recently several investigators have reported that depression of renin activity is not specific for primary hyperaldosteronism (14 18 21 23 29 34) The specificity is not augmented by the use of procedures stimulating renin activity (29 33) Since renin appears to be the most important trophic factor regulating aldosterone secretion (17) it has been found of interest to measure corresponding values of plasma renin activity and aldosterone secretion rate (ASR) in groups of normal subjects and hypertensive patients of different types. The aim was to establish the correlation between the two parameters and to evaluate the significance of the relationship in distinguishing between primary and secondary aldosteronism.

## MATERIAL

*Normal Individuals* (Table I)

Fourteen patients, 11 males, three females, age 16–70. The patients were hospitalized for minor conditions unrelated to the cardiovascular or renal system. In five patients, only ASR was measured.

### *Hypertensive Patients*

All patients had normal excretion of vanillylmandelic acid, ketosteroids, and ketogenic steroids. No patients exhibited signs of cardiac incompensation.

1. *Essential hypertension* (Tables II and III)

Twelve males and five females, age 2? hypertension as defined by grade IV (Keith-Wagener) was ...

Table I *Aldosterone secretion rate in normal subjects*

| | | Age | ASR |
|---|---|---|---|
| Men | 11 | Mean 49 | Mean 97 ± 56 (S D) |
| Women | 3 | Range | ± 15 (S E.M ) |
| | | 16–70 | Range 19–223 |

ASR = aldosterone secretion rate

Symptomatic hypertension was excluded by the following examinations urinary microscopy serum-creatinine urea infusion urography renal arteriography (if indicated) There was no history of kidney disease in any of the cases. Some of the patients were referred to the department because spontaneous or easily induced hypokalaemia suggested primary hyperaldosteronism. This diagnosis was excluded by the finding of PRA > 15 ng/10 ml 4 h (normal mean value 15 ± 2 (S.E M.) (29) or the finding of ASR < 200 μg (normal range – normal mean + 2 × S D = 50–200 see Table I)

II *Renovascular hypertension* (Table IV)

Eight patients, four males and four females, age 45–58 In all eight cases renal arteriography showed a significant renal artery stenosis on one or both (case 84) sides. In all cases except case 51 urea infusion urography showed abnormalities. In case 51 renal arteriog showed left renal artery stenosis with numerous discalibrations of the arterial tree Abdominal showed similar changes in the intestinal vessels. It was concluded that this patient suffered from a disseminated arterial disease also involving the renal vessel and thereby causing hypertension. The surgical results in this group are indicated in Table IV

III. *Parenchymatous renal hypertension* (Table V)

Two patients, one male one female aged respectively 26 and 57 The kidney disease antedated the hypertension by several years. A pronounced destruction of the renal parenchyma had been demonstrated in both cases (biopsy case 26 unilateral nephrectomy case 45)

IV *Primary hyperaldosteronism* (Table VI)

Five patients, three males, two females, age 43–65 Renal disease was excluded as indicated under *I* Four of the patients were admitted because of spontaneous or easily induced hypokalaemia (cases 7 17 47 and 93) The fifth patient (case 79) was consistently normokalaemic. The hypertension was in no case accelerated or malignant. The patients were assigned to this group if PRA was < 15 ng and ASR > 200 μg/24 h. This combination of measurements was found in at least one pair of observations. All five patients subsequently underwent explorative laparotomy The pathological findings and operative results are indicated in Table VI

## METHODS

Plasma renin activity (PRA) was measured a.m. Boucher et al. (5) slightly modified by Nielsen and Møller (28) The coefficient of variation is ± 12 % The results are expressed as ng angiotensin/10 ml plasma/4 h incubation. Aldosterone secretion rate (ASR) was measured by the method of Kliman and Peterson (20) Plasma sodium and potassium were determined by flame photometry The normal individuals received no drug treatment prior to the investigation and were on an unrestricted salt intake. In all hypertensive patients antihypertensive treatment was discontinued seven days before investigation. All patients received normal hospital diet with an unrestricted salt intake or a balanced diet with a known, normal content of

Table II. *Essential non malignant hypertension*

| Case | Sex | Age | BP | FH | Plasma $Na^+$ | Plasma $K^+$ | Serum creatinine | PRA | ASR |
|---|---|---|---|---|---|---|---|---|---|
| 2 | ♀ | 46 | 200 130 | II | 142 | 3 9 | 1 3 | 36 | 94 |
| 10 | ♂ | 63 | 180 120 | II | 144 | 3 5 | 1 1 | 27 | 1[illegible]4 |
| 12 | ♂ | 44 | 175 120 | II | 142 | 3 9 | 1 3 | 13 | 103 |
| 20 | ♂ | 54 | 160 110 | III | 140 | 4 0 | 0 9 | 0 | 69 |
| 26 | ♂ | 58 | 190 110 | I | 143 | 3 4 | 1 0 | 26 | 310 |
| 49 | ♀ | 41 | 150 100 | 0 | 145 | 3 6 | 1 1 | 27 | 206 |
| 52 | ♀ | 39 | 200 100 | 0 | 146 | 3 8 | 1 3 | 48 | 330 |
| 60 | ♀ | 29 | 160 110 | II | 141 | 3 4 | 1 0 | 31 | 231 |
| 62 | ♀ | 62 | 180 105 | 0 | 145 | 3 7 | 1 0 | 0 | 10[illegible] |
| 63 | ♂ | 61 | 170 110 | II | 140 | 3 4 | 0 7 | 4 | 46 |
| 66 | ♂ | 49 | 160 120 | III | 144 | 3 5 | 1 5 | 0 | 79 |
| 67 | ♂ | 35 | 250 150 | II | 143 | 3 7 | 1 0 | 32 | 396 |
| 77 | ♀ | 35 | 160 110 | II | 146 | 3 7 | 1 0 | 23 | 242 |
| 86 | ♀ | 72 | 230 110 | III | 140 | 4 0 | 1 0 | 0 | 29 |

Mean ASR 188 ± 31 (S E.M )
FH = fundus hypertonicus. PRA = plasma renin activity ASR = aldosterone secretion rate The blood pressures indicated are the average supine values of the seven days before measurement of PRA and ASR Plasma $Na^+$ and plasma $K^+$ were determined concomitantly with PRA.

le III *Essential malignant hypertension*

| Sex | Age | BP | FH | Plasma Na+ | Plasma K+ | Serum creatinine | PRA | ASR |
|---|---|---|---|---|---|---|---|---|
| ♂ | 45 | 150/100 | IV | 139 | 3 0 | 1 0 | 245 | 518 |
| ♀ | .0 | 190/140 | IV | 138 | 3 4 | 1 8 | 210 | 701 |
| ♂ | 48 | 170/120 | IV | 144 | 3 5 | 1 5 | 23 | 319 |

n ASR 513 ± 110 (S E M )
= fundus hypertonicus PRA = plasma renin activity ASR = aldosterone secretion rate The blood pressures indicated are average supine values of the seven days before measurement of PRA and ASR Plasma Na and plasma K were deter ed concomitantly with PRA

ium and potassium (see Tables) All blood samples e obtained in the morning (8–10 a.m) After 45 min recumbent position the time required for basal renin ues to become established 18 ml of peripheral venous od was drawn in one .0 ml syringe containing 2 ml 3 8 Na-citrate as anticoagulant The blood was trans red to a siliconised 50 ml Erlenmeyer flask immersed ice water Within one hour the blood was centrifuged 0 C at 3000 r p m for 15 min The plasma was arated and kept at −.0 until renin activity was ermined In another syringe blood was drawn for determination of plasma sodium and potassium Imme diately after the blood was drawn approximately 10 $\mu$C H aldosterone was injected i v for the measurement of ASR. The urine was collected for .4 h from 7 a.m to 7 a m the following day Following hydrolysis at pH 1 for .4 hours duplicate samples of the urine were ex tracted, chromatographed in three systems and ASR determined by a double isotope derivative technique using C acetic anhydrate of known specific activity for the formation of double labelled ?1 monohydroxy aldoste rone The final samples were counted in a two channel

ble IV *Renovascular hypertension*

| se | Sex | Age | BP | FH | Plasma Na+ | Plasma K+ | Serum creatinine | PRA | ASR | Pathological findings | Operation and results |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ♂ | 56 | .00/1.0 | II | 140 | 4 5 | 1 3 | 78 | 337 | Sten a ren dx | Not operated |
| | ♀ | 49 | 200/120 | II | 140 | 3 2 | 0 8 | 26 | 200 | Sten a ren dx | Died before operation |
| | ♂ | 54 | 185/115 | III | 141 | 3 6 | 1 3 | 109 | 390 | Sten a ren s n | Reconstr of ar tery Now normotensive |
| | ♀ | 45 | 195/100 | II | 140 | 3 6 | 1 1 | 35 | 369 | Sten a ren sin | Not operated (see text) |
| | ♀ | 58 | 220/120 | II | 140 | 3 4 | 0 9 | 26 | 242 | Sten a ren sin | Reconstr of ar tery Im proved (BP → 160/100 FH II → FH 0) |
| | ♀ | 52 | 200/120 | II | 141 | 3 6 | 1 6 | 27 | 180 | Sten aa ren bilat | Reconstr of ar tery Now normotensive |
| | ♂ | 54 | 250/140 | IV | 127 | 3 3 | 1 2 | 222 | 326 | Sten a ren sin | Reconstr of ar tery Now normotensive |
| | ♂ | 53 | .00/130 | IV | 141 | 3 8 | 1 0 | 51 | 248 | Sten a ren dx | Reconstr of ar tery Not im proved (Re stenosis) |

ean ASR 87 ± 28 (S E M )
H = fundus hypertonicus PRA = plasma renin activity ASR = aldosterone secretion rate The blood pressures ndicated are e average supine values of the seven days before measurement of PRA and ASR Plasma Na and plasma K were deter ined concomitantly with PRA

Table V *Parenchymatous renal hypertension*

| Case | Sex | Age | BP | FH | Plasma Na | Plasma K | Serum creatinine | PRA | ASR | Kidney disease | Operation and results |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 26 | ♀ | 26 | 180 120 | 0 | 138 | 3 7 | 1 3 | 49 | 223 | Sarcoidosis of the kidney | Not operated |
| 45 | | 57 | 190 110 | II | 143 | 4 1 | 0 8 | 0 | 84 | Unilateral (left) contracted kidney | Nephrex, s.a. now normotensive |

Mean ASR 154 ± 0 (S.E.M.)
FH = fundus hypertonicus. PRA plasma renin activity ASR aldosterone secretion rate The blood pressures indicated are the average supine values of the seven days before measurement of PRA and ASR Plasma Na and plasma K⁺ were determined concomitantly with PRA

liquid scintillation counter (Packard Tricarb) Duplicates varied 10 % or less, or were discarded. Corrections were made for quenching by using an internal standard. In six patients with essential non-malignant hypertension, PRA and ASR were measured after seven days of salt loading (c 200 mEq sodium per day) In three patients with primary hyperaldosteronism PRA and ASR were also measured after salt loading for a corresponding period.

## RESULTS

1 *Aldosterone secretion rate (ASR)*

*Essential non-malignant hypertension* (14 patients Table II) Mean ASR was 188 ± 31 μg per 24 h (S.E.M.) which was significantly higher than in 14 normal subjects whose ASR averaged 97 ± 15 ($n = 27$ $t = 2.6$ $p < 0.02$) (Fig. 1) In seven patients ASR was higher than 200 μg, the upper limit of the normal range

*Essential malignant hypertension* (3 patients Table III) Mean ASR was 512 ± 110 which was significantly higher than in essential non-malignant hypertension ($n = 16$ $t = 2.8$ $p < 0.02$) (Fig. 1)

*Reno-vascular hypertension* (8 patients Table IV) Mean ASR was 287 ± 28 which was significantly

Table VI *Primary hyperaldosteronism*

| Case | Sex | Age | BP | FH | Investigation no | Plasma Na | Plasma K | Serum creatinine | PRA | ASR | Pathological findings | Operation and results |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | ♂ | 54 | 175 130 | II | 1 | 13 | 4 0 | 1 2 | 8 | 130 | Bilateral nodular hyperplasia | Right adrenalectomy No improvement |
| | | | | | 2 | 143 | 3 2 | — | 18 | 258 | | |
| 17 | ♀ | 52 | 225 135 | II | 1 | 146 | 2.8 | 1 3 | 10 | 453 | Adenoma in the left adrenal gland | Left adrenalectomy No improvement |
| | | | | | 2 | 147 | 3 2 | – | 0 | 1464 | | |
| 47 | | 43 | 1 0 110 | II | 1 | 145 | 1 8 | 0 8 | 4 | 1937 | Adenoma in the left adrenal gland | Left adrenalectomy Cured |
| 79 | ♀ | 56 | 180 115 | II | 1 | 141 | 3 7 | 1 2 | 11 | 1310 | Adenoma in the left adrenal b lat. nodular hyperplasia | Left adrenalectomy No improvement |
| 93 | | 65 | 160 110 | II | 1 | 148 | 2.1 | 1.0 | 0 | 923 | Nodular hyperplasia. Only right gland explored | Right adrenalectomy No improvement |

Mean ASR 925 ± 256 (S.E.M.)
FH = fundus hypertonicus. PRA = plasma renin activity ASR = aldosterone secretion rate The blood pressures indicated are the average supine values of the seven days before measurement of PRA and ASR Plasma Na and plasma K⁺ were determined concomitantly with PRA.
Studies of PRA and ASR carried out on balanced diet of 60 mEq Na and 70 mEq K a day (cases 7 17 and 47) or on unrestricted salt intake (cases 79 and 93)

higher than in essential non malignant hypertension ($n=21$ $t=2.4$ $p<0.025$) (Fig. 1)

*Parenchymatous renal hypertension* (2 patients, Table V) Case 26 had a significantly increased ASR while case 45 had a normal ASR. This patient was treated by unilateral nephrectomy and cured of the hypertension.

*Primary hyperaldosteronism* (5 patients, Table VI). Mean ASR was $925 \pm 256$ which was significantly higher than in essential non-malignant hypertension ($n=18$ $t=2.9$ $p<0.01$)

2. *The correlation between ASR and eye ground changes plasma potassium concentration and PRA*

Fig 2 demonstrates the lack of correlation between eye ground changes and ASR in the group of essential hypertensive patients.

Fig. 3 demonstrates the rather weak correlation between plasma potassium concentration and ASR ($r=-0.63$ $p<0.001$)

Figs. 4 and 5 demonstrate the correlation between PRA and ASR for normal subjects and hypertensive patients in the above mentioned groups excluding primary hyperaldosteronism. For PRA < 50 ng/10 ml/4 h the correlation may be described by a straight line ($r=0.83$ $p<0.001$) (Fig. 4) If PRA values $\geq 50$ ng are included the correlation appears to be curvilinear (Fig. 5) The values of PRA and ASR for patients with primary hyperaldosteronism are also plotted in Fig. 4

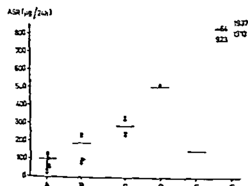



*Fig 1* ASR in the different groups investigated. $A$ = normal subjects. $B$ = essential non-malignant hypertension. $C$ = renovascular hypertension. $D$ = essential malignant hypertension. $E$ = parenchymatous renal hypertension. $F$ = primary hyperaldosteronism.

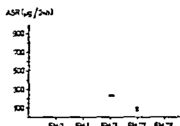



*Fig 2* The correlation between grade of eye-ground changes and ASR in patients with essential hypertension.

(indicated by ◡) They clearly constitute a different population. As indicated in Table VI solitary adenomas were found in two patients (cases 17 and 47) Unilateral adrenalectomy resulted in cure of the hypertension and a return of the electrolyte abnormalities to normal in case 47 whereas no improvement was obtained in case 17 Measurements of PRA and ASR were repeated 6–8 months after operation. In case 17 PRA and ASR were respectively 0 and 3.6 In case 47 the two values were 32 and 73 The three other patients had adenomatous hyperplasia of the adrenals. Unilateral adrenalectomy did not improve the condition.

3 *The effect of salt loading on PRA and ASR in non malignant essential hypertension and primary hyperaldosteronism*

Salt loading depressed PRA and ASR in three of the six non-malignant essential hypertensive patients investigated (Fig. 6) In the three non-reacting patients PRA and ASR were already low before salt loading. There was no difference

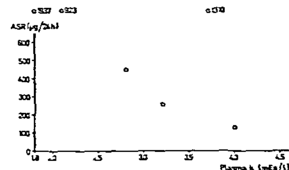



*Fig 3* The correlation between the plasma potassium concentration and ASR. ○ = primary aldosteronism. ● = all other hypertensives.

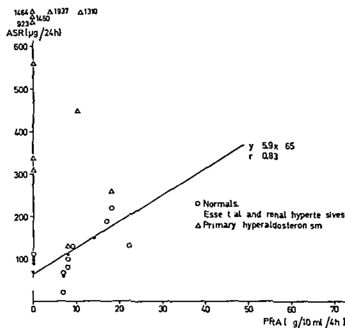



*Fig 4* The correlation between PRA and ASR in normal subjects and all patients investigated with PRA < 50 ng/10 ml/4 h The regression line for normal subjects and patients with essential and renal hypertension is indicated

in sodium excretion before salt loading in the two ups

Salt loading depressed ASR significantly in two of three patients with primary hyperaldosteronism (Fig 7) but the normal range was not reached In the third salt loading caused a significant increase in ASR In all three patients PRA was low or undetectable already prior to salt loading A comparison between Table VI and Fig 7 demonstrates that potassium loading did *not influence ASR in case 47*

## DISCUSSION

The augmented mean ASR of renovascular and essential malignant hypertension is in agreement with the findings reported by other groups (malignant essential hypertension (13 16 23 30) renovascular hypertension (1 23)) It is well known that ASR may be increased in parenchymatous renal hypertension (9 22) More surprising is the demonstration of an increased mean ASR in non malignant essential hypertension In absolutely *benign essential hypertension* Laragh et al

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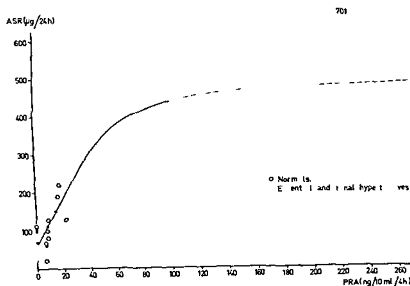



*Fig 5* The correlation between PRA and ASR for normal subjects and patients with hypertension, primary hyperaldosteronism *excluded*.

(23) and George et al (16) found a normal ASR *Laragh et al found that ASR increased with the* severity of the hypertensive eye ground changes In the present investigation no such correlation was found (Fig 2) The high frequency of hypokalaemia in the group of essential non malignant hypertensive patients in the present material (36%) can hardly explain the elevated mean ASR Laragh s patients are comparable to ours with regard to the frequency of hypokalaemia (32 % (25)) but in neither is there a good correlation between plasma potassium concentration and ASR (Fig 3) In contrast George et al (16) found a good correlation between these two parameters all patients with a plasma potassium concentration <3 5 mEq/l had increased ASR.

For normal subjects essential and renal hypertensive patients a rather strong correlation exists between PRA and ASR It is rectilinear over a considerable range of PRA values Laragh et al (23) found a not very pronounced correlation between PRA and ASR in groups of patients very similar to ours In a corresponding group Weinberger et al (34) found a good correlation ($r = 0.80$ $p < 0.001$) *Veyrat et al (32) indicated* a significant linear correlation between the logarithms of PRA and ASR in normal subjects These findings are in agreement with the concept of renin angiotensin as a major trophic factor in the regulation of aldosterone secretion (17) Accordingly the hyperaldosteronism in these groups of patients may be considered to be secondary

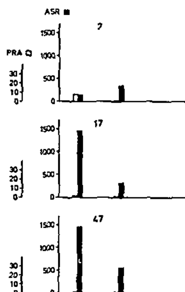



*Fig 7* PRA and ASR before and after salt loading in three patients with primary hyperaldosteronism Case numbers indicated on graph. □ = PRA. ■ = ASR. Prior to salt loading cases 7 and 17 received 80 mEq Na and 70 mEq K a day for one week during salt loading 200 mEq Na and 70 mEq K a day for one week. Case 47 received 80 mEq Na and 193 mEq K a day for one week before salt loading during salt loading 200 mEq Na and 193 mEq K a day The failure of potassium loading during the initial seven-day period to lower ASR is evident from a comparison between ASR in Table VI and Fig 7

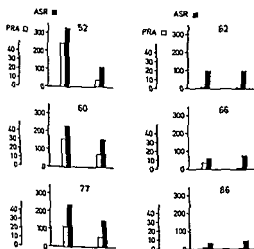



Fig 6 PRA and ASR before and after salt loading in six patients with essential non malignant hypertension. Case numbers indicated on graph □ = PRA. ■ = ASR.

The group of patients with primary hyperaldosteronism was defined by the criteria of low PRA and high ASR Hence these patients comprise a different population from that previously discussed as indicated in Fig 4 According to current concepts the secretion of aldosterone is regulated by ACTH Na K metabolism and renin The influence of sodium metabolism is predominantly exerted through the renin angiotensin system (17) In the situations investigated in the present work stimulation of aldosterone secretion by ACTH may probably be excluded by the demonstration of normal excretion of ketosteroids and ketogenic steroids Because potassium intake was normal stimulation by the potassium load mechanism can be excluded (7) Since PRA was low an elevated ASR can only be attributed to autonomous hypersecretion of aldosterone Of the five cases in this group only one can be considered to be a case

of classical primary hyperaldosteronism (case 47) although case 17 also may be so failure of operation to bring about recovery being due to the *existence of another adenoma in the remaining* gland In the three remaining patients the values for PRA–ASR also indicated primary hyperaldosteronism but at operation the adrenals showed *adenomatous hyperplasia This type has been* described frequently in recent years (6 15 19 25 31) Probably they represent a type of secondary hyperaldosteronism—secondary to factors *other than renin New and Peterson (26) have* described a case of hyperaldosteronism with increased plasma ACTH levels most likely secondary to an enzymatic defect in the adrenal gland *As mentioned above ACTH may be excluded* in our and most other cases but other as yet unknown factors may stimulate aldosterone in such cases as postulated by Davies et al (15) *It is also possible however that our method of* measuring plasma renin fails in certain patients e g due to the presence of in vitro but not in vivo inhibitors

*Certain evidence suggests that aldosterone pro*duction may not be completely autonomous even classical Conn s syndrome For example case , demonstrates a pronounced reduction in ASR _ *salt loading This is in agreement with* findings of Baulieu et al (2) but at variance with findings of Biglieri and Forsham (3) and Lauler et al (24) Thus Biglieri and Forsham (3) found *no change in ASR during salt loading in his pa*tients with primary hyperaldosteronism but stated that ASR was influenced by ACTH blood volume changes and potassium loading This ef*fect of ACTH has been confirmed by Newton and* Laragh (27) while the influence of potassium loading has been confirmed by Cannon et al (7) In case 47 potassium loading did not cause any increase in ASR (Table VI and Fig 7)

Three patients with essential non malignant hypertension showed no suppression of ASR when sodium intake was doubled These patients ini*tially had low PRA and ASR It is conceivable* that the ASR measured before salt loading in these patients was the basal (endogenous) secretion rate corresponding to a minimal stimulation by renin angiotensin If acting with renin it is to be expected that salt loading has no effect on ASR when PRA is already minimal The practical consequences of these findings are that failure of salt loading to cause depression of ASR does not unequivocally suggest adrenal autonomy and hence primary hyperaldosteronism with normal ASR *The existence of primary hyperaldosteronism* with ASR in the normal range was predictable and one case is in fact described by Biglieri et al (4) This case was diagnosed on the basis of a *constant ASR before and after salt loading and* mineralocorticoid administration According to the above mentioned findings in essential hypertension this criterion does not appear to be sufficient *At present the best criterion for making a diag*nosis of primary hyperaldosteronism appears to be a PRA/ASR ratio not corresponding to the correlation line for other hypertensive patients *and normal subjects (Fig 4) However our results* demonstrate that an abnormal PRA/ASR ratio does not permit differentiation between primary hyperaldosteronism due to adenoma and ade*nomatous hyperplasia*

## CONCLUSION

A good correlation between PRA and ASR has been demonstrated in normal subjects and the majority of hypertensive patients investigated The group of hypertensive patients not showing this correlation comprises cases of classical Conn s syndrome and cases with adenomatous hyperplasia of the adrenal gland It is suggested that deviation from this correlation line is indicative of primary hyperaldosteronism *At present it is* not possible to differentiate between simple aldosteronoma capable of cure by operation and patients with adenomatous hyperplasia not profiting *by unilateral adrenalectomy*

## ACKNOWLEDGEMENTS

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## REFERENCES

1 Barraclough M A Bacchus, B Brown J J Davies, D L Lever A F & Robertson, J I S Plasma renin and aldosterone secretion in hypertensive patients with renal or renal artery lesions. Lancet 2 1310 1965

2. Baulieu E E Robel, P Siguier F & Jayle M F. Metabolic observations in a case of pure primary aldosteronism. J clin Endocr 19 1081 1959

3 B..uen, E. G & Forsham, P H. Studies on the extracellular fluid and the responses to various stimuli in primary aldosteronism Amer J Med. 30 564 1961

4 BigLen, E. G Salton, P E., Jr Kronfield, S J & Deck, J B Primary aldosteronism with unusual secretory pattern. J clin. Endocr 27 715 1967

5 Boucher R., Veyrat, R., de Champlain, J & Genest, J New procedures for measurement of human plasma angiotensin and renin activity levels. Canad med. Ass. J 90 194 1964

6 Brown, J J., Chinn, R. H., Davies, D L., Duster dieck, G., Fraser R., Lever A. F., Robertson, J I S., Tree M & Wiseman, A. Plasma electrolytes, renin and aldosterone in the diagnosis of primary hyper aldosteronism. Lancet 2. 55 1968

7 Cannon, P J Ames, R. P & Laragh, J H.. Relation between potassium balance and aldosterone secretion in normal subjects and in patients with hypertensi e or renal tubular disease J clin. Invest. 45 865 1966

8 Conn, J W Plasma renin activity in primary aldosteronism. J Amer med. Ass. 190· 222, 1964

9 Conn J W & Conn, E. S Primary aldo teronism versus hypertensi e disease with secondary aldosteronism. Recent Progr Hormone Res. 17 389 1961

10 Conn, J W Cohen E. L. & Rovner D R. Suppression of plasma renin activity in primary aldosteronism. J Amer med. Ass. 190· 213 1964

11 Conn, J W., Cohen E. L., Rovner D R. & Nesbit, R. M Normokalemic primary aldosteronism. J Amer med. Ass. 193 200 1965

12. Conn, J W., Rovner D R., Cohen, E. L. & Nesbit, R. M.. Normokalemic primary aldosteronism. J Amer med. Ass. 195 21 1966

13 Cope C L., Harwood, M & Pearson, J Aldosterone secretion in hypertensi e diseases. Brit. Med. J 1 659 196..

14 Creditor M C & Loschky U K. Plasma renin activity in hypertension. Amer J Med. 43 371 1967

15 Davies, W W., Newsome H. H., Wright, L. D V Jr Hammond, W G., Easton, J & Bartter F C Bilateral adrenal hyperplasia as a cause of primary aldosteronism with hypertension, hypokalemia and suppressed renin activity Amer J Med. 42. 64.. 1967

16 George J M Gillespie, L. & Bartter F C Aldosterone secretion in hypertension. Amer Med. 69 693 1968

17 Gross, F Control of aldosterone secretion by the renin-angiotensin system and by corticotropin. Advanc intern. Med. 14 ..81 1968

18 Gunnells, J C Jr Grim, C. E., Robinson, R. R. & Wildermann, N M Plasma renin in healthy subjects and patients with hypertension. Arch. intern. Med. 119 23.. 1967

19 Katz, F H. Primary aldosteronism with suppressed plasma renin activity due to bilateral nodular adrenocortical hyperplasia. Amer J Med. 67 1035 1967

..0 Kliman, B & Petersen, R. E.. Double isotope derivation assay of aldosterone in biological extracts. J biol. Chem. 235 1639 1960

21 Kuchel, O., Fishman, L. M., Liddle, G W & Mi hel akis, A. M Effect of diazoxide on plasma renin activity in hypertensive patients Amer J Med 67 791 1967

2.. Laragh, J H., Ulick, S., Januszewicz, V., Deming, Q B Kelly W G & Lieberman, S Aldosterone secretion in primary and malignant hypertension. J clin. Invest. 39 1091 1960

23 Laragh, J H., Sealy J E. & Sommers, S C. *Patterns of adrenal secretion and urinary excretion* of aldosterone and plasma renin activity in normal and hypertensive subjects. Circulat Res. Suppl. 1 158 1966

..4 Lauler D P., Hickler R. B & Thorn, G W Diagnostic criteria for clinical diagnosis of primary aldosteronism. J clin. In est. 45 1035 1966

25 Ledingham, J G G., Bull, M. B & Laragh, J H.. *The meaning of aldosteronism in hypertensi e disease.* Circulat Res. Suppl. 2 177 1967

..6 New M J & Peterson, R. E. A new form of congenital adrenal hyperplasia. J clin. Endocr 27 300 1967

27 Newton, M A. & Laragh, J H Effect of corticotropin on aldosterone excretion and plasma renin in normal subjects, in essential hypertension and primary aldosteronism J clin. Endocr ..8 1006 1968

..8 Nielsen I & Møller I Simultaneous determination of renin activity and angiotensin concentration le els in human plasma. Acta med. scand. 18.. ..63 1967

9 — The postural plasma renin activity response in essential hypertension and primary hyperaldosteronism Acta med. scand. 186 ..65 1969

30 Sambhi, M. P., Beck, J C & Venning, E. H. Malignant hypertension and aldosterone secretion Amer J Med. 35 251 1963

31 Sutherland, D J A., Ruse J L & Laidlaw J C Hypertension, increased aldosterone secretion and low plasma renin activity relieved by dexamethasone Canad. med. Ass. J 95 1109 1966

32. Veyrat, R., Muller A F & Mach R. S Les hyperaldosteronismes secondaires. Schweiz. med. Wschr 98 65 1968

33 Warter J., Schwartz, J., Bloch, R., Desaulles, E., Velley J & Imbs, J L Variations posturales des taux de rénine plasmatique chez l'homme normal et chez l'hypertendu Presse med. 74 ..0..4 1966

34 Weinberger M H., Dowdy A. J., Nokes, G W & Luetscher J A. Plasma renin activity and aldosterone secretion in hypertension patients during high and low sodium intake and administration of d uretic J clin. Endocr ..8 359 1968

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# VISCERAL CHANGES IN SEVERE HYPERTENSION AND THEIR RESPONSE TO DRUG TREATMENT

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*Abstract* On the basis of a series of 112 patients with severe hypertension, treated in an outpatient clinic for an average period of 3.5 years, the most important visceral changes are described on the basis of retinal findings, electrocardiogram, heart volume and serum creatinine The effect of treatment on these objective symptoms is correlated to the degree of reduction in blood pressure obtained. Reduction in the mean blood pressure to ≤133 of the normal for the age produced a considerable regression of the retinal and cardiac changes caused by hypertension, whereas in about 90% of these patients the serum creatinine concentration did not increase during the period of treatment. A further reduction in blood pressure to ≤120% of normal produced corresponding but not better results. In patients with poorer regulation of blood pressure (blood pressure >133% of normal) the results however were considerably less favourable The renal function at commencement of treatment was found to be of great importance for the prognosis.

During drug treatment of hypertension it appears most natural to use the effect of treatment on the blood pressure as a guide However since it is not the actual blood pressure readings but the visceral changes which afford the indication for treatment, the long range effects on these changes will be the most important factor in the evaluation of the efficiency of treatment

In the present paper describing a group of patients with severe hypertension the most important visceral changes and their response to reduction of blood pressure by drug therapy will be discussed We have been particularly interested in the relationship between the degree of reduction in blood pressure induced and the regression of visceral changes inter alia with the object of establishing the optimum intensity of treatment

## MATERIAL AND METHODS

The patients were outpatients of our hypertension clinic which was established in 1958 From 1958–1968 ?48 patients with hypertension were followed up here for shorter or longer periods. Before initiating treatment on an outpatient basis, all patients were admitted to hospital for an aetiological and clinical diagnosis, and during the follow up period we aimed at having all patients admitted to hospital for brief annual check ups.

The patients were selected for the present study on the following criteria.

1 Patients with grade IV retinal changes (Keith–Wagener classification)

2 Patients with grade III retinal changes. Of these patients those with fresh exudates are classified as grade III+ the remainder as grade III− (see below)

3 Patients with grade I–II retinal changes and a mean blood pressure increased by more than 50% of the values normal for the age. (Normal values according to Hamilton et al (4))

4 All the patients have been followed up on an out patient basis for more than six months, most of them for considerably longer periods. The average period of follow up was 3.5 years.

The material which subject to the above criteria, comprised 112 patients was selected with the object of including a suitable number of patients with the highest possible incidence of visceral changes. Table I presents the clinical data which we investigated during the present study In 94 of the patients, according to our results, the hypertension must be characterized as essential. The following presumed aetiological factors were observed chronic glomerulonephritis (7 patients) chronic pyelonephritis (5 patients) stenosis of the renal artery (4 patients) and diabetic nephropathy (? patients)

The treatment was guided by monthly consultations in the outpatient clinic during which the blood pressure was determined with the patient lying, and erect after 10 min rest. The blood pressure values are given as mean blood pressure (diastolic pressure + ⅓ of the blood pressure amplitude) in the lying position.

Of course drug treatment has changed somewhat during the last decade but the main rule has been that practically all patients were given thiazide as basic therapy As supplementary treatment, during recent years, we have in particular employed alpha methyl-dopa and, in the most severe cases, we have also given a sympathicus blocker most often betanidine Hydralazine has been used to a certain extent Furthermore for reasons of investigation, many of the patients have transitorily been given a more atypical treatment

Unless contraindicated by complicating diseases, we

A

Table I *Clinical data of 112 patients with severe hypertension*

| Retinal changes (grade) | Sex | No of pats | Age | Treatm nt (y ) | MBP[a] Average (mm Hg) | Serum creatinine (mg/100 ml) | Heart volume | | ECG | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | N | N | Normal | Abnormal |
| IV | ♂ | 7 | 51 | 1 6 | 173 | 2 4 | 2 | 5 | 1 | 6 |
| | ♀ | 7 | 33 | 4 6 | | 2 1 | 4 | 3 | 2 | 5 |
| III+ | ♂ | 16 | 48 | 3 3 | 163 | 1 8 | 5 | 11 | 4 | 12 |
| | ♀ | 3 | 29 | 3 7 | | 1 9 | 3 | 0 | 1 | 2 |
| III− | ♂ | 23 | 50 | 4 3 | 166 | 1 5 | 8 | 15 | 6 | 17 |
| | ♀ | 14 | 49 | 3 0 | | 1 2 | 3 | 11 | 4 | 10 |
| I–II | ♂ | 20 | 43 | 3 6 | 159 | 1 3 | 12 | 8 | 4 | 16 |
| | ♀ | 22 | 42 | 3 6 | | 1 2 | 13 | 9 | 9 | 13 |

[a] MBP = mean blood pressure

aimed at securing normal blood pressure readings However as is known this is not always possible and generally a certain reduction in blood pressure was obtained the degree of which was dependent on the side effects the co-operation of the patient and the care exerted by the physician

his variation in the results of treatment forms the s of a completely arbitrary classification of the patients o three treatment groups according to the classifcation in Table II The normal values in this table are graded according to Hamilton et al (4) the lower hypertensive limit according to Masters et al (9) The treatment group good represents cases in which the blood pressure readings in the supine position during the greater part of the treatment period were increased by less than 20 of those normal for the age In the treatment group acceptable the corresponding value was 33 / whereas the blood pressure level in the group unacceptable was higher Table III shows the classification of the patients into these three groups and the average mean blood pressure values before and during treatment for the four fundus groups.

The four parameters investigated during this study were

1 Ophthalmoscopy for determination of the degree of retinopathy (Keith Wagener) A division of grade III retinal changes into two subgrades is not generally made However this group appears so inhomogeneous that we find it justifiable to divide the cases into those with fresh exudates (premalignant changes) and those with less severe older changes.

2. *Calculation of the heart volume in relation to the* body surface area, on the basis of measurements of the heart shadow on chest X ray from the equation

$$\frac{0\,42(L\ B\ Da)}{\text{body surface area (m}^2)} \qquad (1)$$

where $L$ and $B$ represent the long and the broad diameter respectively of the elliptical cardiac silhouette in the frontal view at a focus film distance of 1 5 m, and $Da$ (absolute depth diameter) is the greatest diameter perpendicular to the longitudinal axis of the heart in the sagittal view

Most workers employ the greatest depth diameter in the horizontal plane (direction of the ray) However we found a better reproducibility of $Da$ as it is not affected by any oblique position of the patient in the sagittal plane during exposure Consequently our normal values—4 0 ml/m in females 450 ml/m in males—are somewhat lower than those generally reported because we applied the most widely used multiplication factor (0 42)

However the efficacy of this method is due to the fact not that it provides absolute figures but that it affords a good possibility for assessing the relative size of the heart in the same patient at different times The inaccuracy of the method is usually estimated at 10 or somewhat higher (12)

3 Electrocardiography—three limb leads and the nd and 4th precordial leads

4 Serum creatinine A change in serum creatinine *concentration of 0 4 mg/100 ml was chosen* as the lowest figure expressing a genuine change in this value

## RESULTS

### 1 *Retinal changes*

Table IV shows changes in the retinal findings caused by hypertension during treatment in 111 patients (one patient had to be excluded because no check up results were available) Independent of blood pressure readings during treatment the

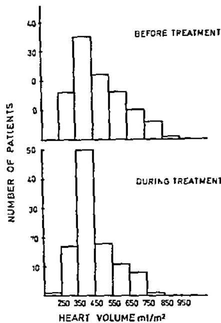



*Fig 1* Heart volume before and during treatment (106 patients)

Table II *Blood pressure limits for the various treatment groups*

| Age | Normal | Lower hypertension (limit) | Good (MBP) | Acceptable (MBP) |
|---|---|---|---|---|
| 30-39 | 124/76 (92) | 153/98 (116) | 111 | 123 |
| 40-49 | 133/80 (98) | 169/103 (125) | 118 | 131 |
| 50-65 | 149 87 (107) | 183/108 (133) | 128 | 143 |
| Per cent of normal | | 125 | 120 | 133 |

MBP

malignant and premalignant changes disappeared in all patients and in no case was the retinopathy aggravated Out of the 14 patients in whom the eyeground changed to or remained fundus grade III—two (15 %) belonged to treatment group "good" five (35 %) to group "acceptable" and seven (50 %) to group "unacceptable"

2. *Heart volume*

In six patients it was not possible with any degree of accuracy to determine the heart volume (obesity hydrothorax) and consequently they are excluded from this part of the survey In the remaining 106 patients heart volumes before and during treatment, respectively appear from Fig 1 In 58 of these 106 patients the heart volume was increased prior to treatment. During treatment the number of these patients was reduced to 37 In five of the 48 patients with normal heart volume at commencement of treatment the volume increased during treatment Table V shows the changes in average heart volume in the three treatment groups Each group is divided into patients with normal and enlarged heart before treatment It appears from this table that in the best two treatment groups we succeeded in preventing growth of a normal heart whereas a considerable growth occurred in the group with the poorest regulation of blood pressure Correspondingly a more modest regression was observed in the latter group in cases of heart ectasia

3 *Electrocardiography*

The electrocardiographic findings and the changes that occurred during treatment are presented in Table VI The group with normal ECG includes

Table III *Blood pressure (mm Hg) before and during treatment and the distribution of patients into the three treatment groups*

| Retinal changes (grade) | No of pats. | MBP (mm Hg) Before treatment | MBP (mm Hg) During treatment | Treatment: No of pats per cent Good | Acceptable | Unacceptable |
|---|---|---|---|---|---|---|
| IV | 14 | 173 | 123 | 8 (57) | 3 (21 5) | 3 (21 5) |
| III+ | 19 | 163 | 122 | 10 (53) | 6 (31) | 3 (16) |
| III– | 37 | 166 | 126 | 19 (51) | 10 (27) | 8 (22) |
| I II | 42 | 159 | 123 | 17 (41) | 16 (38) | 9 ( 1) |
| Total | 112 | | | 54 (49) | 35 (31) | 22 ( 0) |

Table IV *Retinal changes before and during treatment (111 patients)*

| Retinal changes (grade) before treatment | No of pats. | Retinal changes during treatment | | | |
|---|---|---|---|---|---|
| | | I–II | III – | III + | IV |
| IV | 14 | 9 | 5 | 0 | 0 |
| III + | 18 | 15 | 3 | 0 | 0 |
| III – | 37 | 31 | 6 | 0 | 0 |
| I–II | 42 | 4. | 0 | 0 | 0 |

patients with left axis deviation but without signs of left hypertrophy in the precordial leads. "Left strain light degree" indicates cases with diphasic or isoelectric T waves in one or more of the typical leads (1st limb lead 2nd limb lead, 4th precordial lead) or distinctly negative T waves in a single lead (in actual practice always the 1st limb lead) The group "left strain, heavy degree" comprises cases with typical strain pattern (definitely negative T wave) in at least two of the typical leads. Slight changes in the T waves might, in a number of cases, be caused by coronary sclerosis rather than hypertension, but no attempt between these factors has been made the high degree of reversibility indicates the changes are mainly hypertensive

During treatment four patients developed changes other than those described. They were two patients with perpetual arrhythmia and two with acute coronary thrombosis. In 28 of the 112 patients the electrocardiographic results must be characterized as unsatisfactory This means that the electrocardiogram either deteriorated or that cases of left strain remained unchanged. Out of these 28 patients ten (36%) were listed under the treatment group "good" 3 (11%) under the group "acceptable" and 15 (52%) under the group "unacceptable" The corresponding percentages for the remaining 84 patients were 52, 38 and 10 respectively

*Serum creatinine*

Table VII shows the serum creatinine concentrations during treatment in the three groups. Since before treatment, the serum creatinine values were on an average higher in the "unacceptable" than in the other two groups, a direct comparison would be misleading, because the prognosis in respect to renal function is presumably poorer the higher the serum creatinine at commencement of treatment. In order to eliminate this source of error the results are shown in Table VIII in relation to the serum creatinine values at commence

Table V *Heart volume (ml/m²) before and during treatment related to blood pressure reduction (106 patients)*

| Treatment group | No of pats. | Heart volume = normal | | | No of pats. | Heart volume > normal | | |
|---|---|---|---|---|---|---|---|---|
| | | Before treatment | During treatment | Decrease | | Before treatment | During treatment | Decrease |
| Good | 24 | 364 | 349 | 15 | 28 | 580 | 485 | 95 |
| Acceptable | 20 | 3 0 | 379 | −9 | 12 | 589 | 458 | 131 |
| Unacceptable | 4 | 351 | 432 | −81 | 18 | 606 | 561 | 45 |

Table VI. *ECG before and during treatment*

| ECG before treatment | No of pats. | ECG during treatment | | | | |
|---|---|---|---|---|---|---|
| | | Normal | Left hypertrophy | Left strain, light degree | Left strain, heavy degree | Other changes |
| Normal | 31 | 29 | 0 | 2 | 0 | 0 |
| Left hypertrophy | 12 | 4 | 7 | 0 | 0 | 1 |
| Left strain, light degree | 34 | 16 | 4 | 7 | 5 | 2 |
| Left strain, heavy degree | 35 | 5 | 4 | 15 | 10 | 1 |

Table VII *Serum creatinine before and during treatment related to blood pressure reduction (mg/100 ml)*

| Treatment group | No of pats | Creatinine before treatment | Creatinine during treatment | | Deaths from uraemia |
|---|---|---|---|---|---|
| | | | Unchanged | Increased | |
| Good | 54 | 1 39 | 49 | 5 | 1 |
| Acceptable | 35 | 1 42 | 31 | 4 | 2 |
| Unacceptable | 23 | 1 82 | 14 | 9 | 5 |

ment of treatment The figures are too low to justify any definite conclusions, although they indicate that also in cases of renal functional impairment an efficient reduction in blood pressure will be of decisive importance

*5 Collective results*

In Table IX we have tried to summarize the results in the three treatment groups the figures in the table showing the percentage of patients with satisfactory results of treatment in respect to the four parameters investigated The criteria for satisfactory results were

*re 1* Grade I–II retinal changes

*re 2* Normal heart volume not increased ⩾10 Increased volume decreased by at least 10%

*re 3* ECG before treatment. Normal or left hypertrophy left strain light degree left strain heavy degree. ECG during treatment. Normal or unchanged normal or left hypertrophy normal left hypertrophy or strain, light degree

*re 4* Serum creatinine unchanged or increased <0 4 mg/100 ml

*6 Causes of death*

A total of 11 patients died during the follow up in the outpatient clinic The causes of death appear in Table X where they are related to the serum creatinine concentrations before treatment The two patients who died from cardiac infarction were men aged 49 and 52 years and were classified under the treatment groups good and "acceptable respectively The patient with cerebral thrombosis was a 65 year-old man The average age of the eight patients who died from uraemia was 42 years average period of treatment 3 7 years

## DISCUSSION

In conformity with what is known from reports on drug treatment of hypertension published during recent years we found it possible by reducing the blood pressure level to favourably influence the visceral changes caused by hypertension (2, 3 5 8 10 13)

The severe retinal changes seem to be the easiest to influence in our material the malignant and

Table VIII *Patients with increasing serum creatinine during treatment related to serum creatinine before treatment (mg/100 ml)*

| Creatinine before treatment | Treatment group | No of pats | Patients with increasing creatinine | No of deaths from uraemia |
|---|---|---|---|---|
| <1 3 | Good | 32 | 0 | 0 |
| | Acceptable | 21 | 1 | 0 |
| | Unacceptable | 11 | 2 | 1 |
| 1 4–1 9 | Good | 17 | 3 | 0 |
| | Acceptable | 7 | 0 | 0 |
| | Unacceptable | 5 | 3 | 0 |
| ⩾2 0 | Good | 5 | 2 | 1 |
| | Acceptable | 7 | 3 | 2 |
| | Unacceptable | 7 | 4 | 4 |

Table IX *Summary of the effect of treatment related to blood pressure reduction (% of patients with satisfying results)*

| Treatment group | Retinal changes | ECG | Heart volume | Serum creatinine |
|---|---|---|---|---|
| Good | 96 | 82 | 79 | 91 |
| Acceptable | 86 | 91 | 78 | 91 |
| Unacceptable | 68 | 35 | 32 | 65 |

Table X *Causes of death related to serum creatinine before treatment (mg/100 ml)*

| Serum creatinine | No of pats | No of deaths | Cause of death |
|---|---|---|---|
| <2 0 | 93 | 2 | Acute myocard infarct |
| | | 1 | Uraemia |
| ≥2 0 | 19 | 1 | Cerebral thrombosis |
| | | 7 | Uraemia |

premalignant changes disappeared irrespective of any reduction in blood pressure

According to our criteria a satisfactory effect on the cardiac symptoms occurred in 80–90 % of the cases but only in patients in whom a pronounced reduction in blood pressure was obtained ... er definitely the most important factor is it was possible at the same time to retard the development or to arrest the progression of a hypertensive nephropathy in almost 90 % of these patients

Perhaps with one exception (8) other authors have not found to a similar degree the considerable reversibility mentioned in particular in the cardiac changes (5 11 13 14) Hence our results seem to be more favourable than most of those previously reported This is presumably due primarily to the advances in drug therapy during recent years but also to the fact that we selected the most severe cases Naturally the high incidence of visceral changes will increase the possibilities of recording favourable results of treatment

A direct comparison between our results and previous reports on this subject is impeded furthermore by the considerable variation in the statements regarding the reduction in blood pressure desired Most frequently a diastolic pressure of 100–110 mm Hg is considered satisfactory (1 3 7) only few authors change their requirements according to the age of the patients and many report readings only in the standing position. In particular this latter practice must be considered unsatisfactory especially in circumstances as in our study which concerns severe cases in which treatment with drugs with a pronounced orthostatic effect is often required On the face of it the ideal requirement must be a complete normalization of the blood pressure and Werko among others emphasizes that this must be the purpose of any therapy (16)

Indeed our criteria for satisfactory regulation of blood pressure are strict and concern solely measurements in the supine position They correspond approximately to those previously stated by Vejlsgard et al (15)

One of the objects of the present investigation was through a rough selection to try to establish the intensity of treatment required to obtain the optimum effect on the visceral changes caused by hypertension Since as mentioned above the retinal changes presented high reversibility even after a very modest reduction in blood pressure in the long run these changes can hardly serve as a suitable guide to the efficiency of the treatment. On the other hand the results of the investigation of the heart volume and electrocardiogram showed considerable differences between the group of patients with the poorest regulation of blood pressure and the two groups which were treated most intensively The results in the two latter groups were not categorically different This was also found by investigating serum creatinine although the groups were not directly comparable because of differences in the starting values As found by other investigators (2 6 10) however there is very little doubt that a thorough regulation of the blood pressure is of decisive importance for the prognosis of the renal function

The fact that no definite difference was observed between the results in the two groups which were treated most effectively indicates that a rigorous demand for normalisation of the blood pressure in these patients is not necessary On the other hand an almost complete normalisation which was obtained in about 50 % of the patients was well tolerated and gave completely favourable results.

## REFERENCES

1 Bath J Pickering D & Turner R. Brit. med. J 4 519 1967

2 Bjork S Sannerstedt R Falkheden, T & Hood B Acta med scand 169 673 1961
3 Dustan, H P Schneeckloth R E Corcoran, A C & Page I H Circulation 18 644 1958
4 Hamilton, M Pickering J A Fraser Roberts J A & Sowry G S C Clin Sci 13 11 1954
5 Harington, M Kincaid-Smith P & McMichael J Brit med J 5158 1959
6 Hood B Aurell, M Falkheden T & Bjork, S Antihypertensive therapy p 370 Springer Berlin 1966
7 Johnston, A. W., Pichard B C N & Rosenheim, M L Lancet 2 659 1964
8 Mackeprang, B Ugeskr Læg 129 673 1967
9 Master A M Garfield, C I & Walters, M B Normal blood pressure and hypertension p 94 Lea & Febiger Philadelphia 195
10 Moyer J H Heider C Pevey K & Ford V R Amer J Med 24 177 1958
11 Rønnov Jessen V Behandling af Hypertensio Arterialis med gangliebloKerende midler Thesis Copenhagen 1957
12 Schinz, H R Baensch, W E Friedl E & Uehlinger E Lehrbuch der Rontgendiagnostik, p 2704 Stuttgart 1952
13 Sturup H & Gruner A Acta med scand 160 251 1958
14 Solvsten, P Ugeskr Læg 1_1 3_6 1959
15 Vejlsgård V Christensen M & Clausen, E Brit med J 1 598 1967
16 Werko L Hypertension, p 140 Almqvist & Wiksell Stockholm 1968

# Congress Announcements

*The 56th Annual Clinical Congress of the American College of Surgeons* will be held in Chicago Illinois October 12–16 1970 Some 14 000 doctors and guests from throughout the world are expected to attend Headquarters hotel the Conrad Hilton

Official registration forms available after June 1 from Mr T E. McGinnis American College of Surgeons 55 East Erie Street Chicago Illinois 60611

*The XI International Congress of Internal Medicine* will be held in New Delhi October 25 to 30 1970

*Topics* Advances in immunology diseases peculiar to tropics medical aspects of population explosion malnutrition

Applications for taking part in the congress should be sent to the Secretariat address V P Chest Institute Delhi University Delhi India

*The VIII Conference of European Dialysis and Transplant Association (E D T A)* will be held in Berlin July 1 to 3 1971

*President* N Alwall M D Lund Sweden

*Congress Office* Dr R Natusch 2nd Medical Dept (Charite) 104 Berlin DDR Schumannstrasse 20–21

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# GLOMERULAR FILTRATION RATE IN PATIENTS WITH SEVERE AND VERY SEVERE RENAL INSUFFICIENCY

*Determined by Simultaneous Inulin Creatinine and ¹²⁵I-Iothalamate Clearance*

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*Abstract* Clearance studies in patients with markedly reduced renal function can be performed with satisfactory accuracy without catheterization of the bladder A reliable infusion technique careful instruction and supervision during the study together with clearance periods of adequate length, are necessary Endogenous creatinine clearance does not provide a reliable expression for glomerular filtration in patients with renal insufficiency and in patients with the nephrotic syndrome, because renal function is consistently overestimated. This overestimation is approximately 25 to 50% in patients with an inulin clearance less than 5 ml/min. ¹²⁵Iothalamate clearance in the same series of patients provided an exact measure of glomerular filtration rate, as the mean ratio $^{C}Io/^{C}In$ was 0.97

In 1926 Rehberg (38) suggested the use of exogenous creatinine clearance for determination of the glomerular filtration rate (G F R ) A few years later Miller and Winkler (30) showed that the exogenous creatinine clearance in man is greater than true glomerular function as measured by inulin clearance In 1937 Popper and Mandel (35) found that endogenous creatinine clearance gave an excellent measure of G F R. This method has since then been the preferred one for routine clinical use because of its obvious advantages. The plasma concentration is constant, the excretion of creatinine is independent of urine volume and diet (1 10) long clearance periods reduce errors of collection, and hydration and catheterization are unnecessary The method provides a good measure of G F R. in individuals with normal kidney function (5 18 21 30 40 46) However with renal insufficiency there is a marked discrepancy between G F R. measured by inulin clearance and by endogenous creatinine clearance (3 9 23 27 30 32, 48) This disagreement is partly due to various chemical procedures for the determination of creatinine (4 5 21 26 29 34) and partly to a changed mechanism of excretion in patients with renal insufficiency (9 27)

Inulin clearance has been the standard method for determination of G F R. since its introduction in 1934 (37 41) because of the physical and physiological properties of inulin (28 31 47) The chemical determination of inulin is, however time-consuming and associated with analytical errors regardless of the method of measurement used (15 22, 24 50)

During recent years isotope techniques have been used for the determination of G F R. (8 13 14 33 42, 43) Common to all of these new substances is the simple way and quantitative exactness with which they can be measured in the blood and urine Nelp et al. (33) in 1964 developed a method for measuring G F R. using radioactive $B_{1_}$ and found a close correlation between clearance for tagged $B_1$ and inulin. Problems associated with the saturation dose of stable $B_1$ prior to measurement of clearance the inconstant binding of tagged $B_1$ to plasma proteins during measurement of clearance and the possibility of exchange with stable $B_{1_}$ in the patient s stores make this method less suitable

An isotope-tagged substance which is not bound to protein was found by Elwood and Sigmann (13 14 42, 43) in iothalamate—a substituted triiodobenzoic acid derivative with a molecular weight of 614 Simultaneous iothalamate and inulin clearance studies have given almost identical results.

Previously published series have however included only a few studies in patients with severely

| Pat no | Sex | Age | Diagnosis | Proteinuria (g/d) | Plasma-creat (mg/100 ml) | No of periods | Average clearance Inulin | Average clearance Creatinine | Average clearance Iothalamate | Clearance ratio Creatinine inulin Average | Creatinine inulin Range | Creatinine inulin S D | Iothalamate inulin Average | Iothalamate inulin Range | Iothalamate inulin S D | Plasma alb (g/100 ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ♂ | 22 | Chr glomer | 1–2 | 26 0 | 3 | 1 64 | 1 78 | 1 59 | 1 08 | 1 04<br>1 12 | 0 041 | 0 97 | 0 96<br>0 98 | 0 010 | 3 4 |
| 2 | ♂ | 44 | Chr glomer | 2 3 | 18 0 | 3 | 1 66 | 1 93 | 1 50 | 1 16 | 1 07<br>1 28 | 0 108 | 0 90 | 0 82<br>0 98 | 0 80 | 4 0 |
| 3 | ♀ | 18 | Chr pyelon | 2 3 | 19 2 | 3 | 1 72 | 2 54 | 1 79 | 1 43 | 1 18<br>1 72 | 0 [illegible]73 | 1 01 | 0 88<br>1 26 | 0 216 | 4 1 |
| 4 | ♂ | 46 | Chr glomer<br>Chr pyelon | 1–2 | 19 5 | 3 | 1 74 | 2 25 | 1 88 | 1 29 | 1 24<br>1 38 | 0 080 | 1 08 | 0 98<br>1 13 | 0 084 | 4 1 |
| 5 | ♂ | 44 | Chr glomer | 2 3 | 17 5 | 3 | 1 82 | 1 99 | 1 70 | 1 12 | 1 07<br>1 21 | 0 078 | 0 94 | 0 89<br>0 99 | 0 050 | 3 9 |
| 6 | | 2 | *Obstr nephr* | 1–2 | 19 5 | 3 | 1 87 | 2 60 | 2 03 | 1 41 | 1 16<br>1 56 | 0 216 | 1 10 | 0 98<br>1 21 | 0 116 | 3 2 |
| 7 | ♀ | 14 | Chr glomer | 3–4 | 29 5 | 3 | 1 90 | 2 46 | 2 16 | 1 28 | 1 04<br>1 40 | 0 210 | 1 14 | 1 08<br>1 22 | 0 070 | 5 2 |
| 8 | ♀ | 18 | Chr pyelon | 2 5 3 5 | 21 3 | 2 | 1 92 | 2 31 | 1 69 | 1 20 | 1 05<br>1 35 | 0 212 | 0 88 | 0 88<br>0 88 | 0 000 | 5 0 |
| 9 | ♂ | 28 | Hered nephr | 1 5 2 0 | 24 5 | 3 | 2 17 | 2 54 | 2 13 | 1 18 | 1 05<br>1 28 | 0 118 | 0 99 | 0 93<br>1 04 | 0 055 | 4 8 |
| 10 | ♀ | 18 | Chr pyelon | 2 5 1 5 | [illegible]1 3 | 3 | 2 21 | 2 50 | 1 92 | 1 15 | 1 03<br>1 35 | 0 174 | 0 88 | 0 86<br>0 90 | 0 020 | 5 0 |
| 11 | ♂ | 64 | *Chr glomer* | 0 2–0 5 | 19 8 | 3 | 2 23 | 3 12 | 2 14 | 1 40 | 1 33<br>1 45 | 0 061 | 0 97 | 0 94<br>0 96 | 0 017 | 3 9 |
| 12 | ♂ | 41 | Chr pyelon | 3 5–6 0 | 16 5 | 3 | 2 31 | 3 16 | 2 47 | 1 39 | 1 27<br>1 49 | 0 110 | 1 06 | 1 03<br>1 11 | 0 040 | 3 0 |
| 13 | ♂ | 47 | Chr glomer | 1 0–2 5 | 16 5 | 3 | 2 3[illegible] | 2 75 | 2 52 | 1 19 | 1 09<br>1 28 | 0 101 | 1 09 | 1 02<br>1 18 | 0 082 | 4 4 |
| 14 | ♀ | 41 | Chr pyelon | 1 2 | 16 3 | 3 | 2 35 | 2 42 | 2 10 | 1 04 | 0 99<br>1 08 | 0 045 | 0 90 | 0 87<br>0 93 | 0 031 | 3 3 |
| 15 | ♂ | 28 | Hered nephr | 3 5 | [illegible] | 3 | 3 15 | 3 21 | 3 07 | 1 03 | 0 93<br>1 11 | 0 092 | 0 98 | 0 93<br>1 03 | 0 050 | 4 7 |
| 16 | ♂ | 19 | *Chr glomer*<br>Nephrotı | 5–10 | 13 5 | 3 | 3 57 | 4 51 | 3 27 | 1 26 | 1 21<br>1 32 | 0 056 | 0 93 | 0 89<br>0 92 | 0 023 | 2 6 |
| 17 | ♂ | 45 | Polycysti k | 0 5–1 5 | 13 0 | 3 | 3 58 | 5 94 | 3 06 | 1 73 | 1 30<br>2 28 | 0 500 | 0 85 | 0 82<br>0 87 | 0 026 | 4 2 |
| 18 | ♀ | 46 | Chr pyelon | 0 4 1 5 | 15 0 | 3 | 3 89 | 5 49 | 3 83 | 1 44 | 1 28<br>1 68 | 0 [illegible]11 | 0 99 | 0 93<br>1 03 | 0 055 | 3 9 |
| 19 | ♀ | 21 | Chr pyelon | 0 3–0 6 | 17 0 | 3 | 3 98 | 3 74 | 3 82 | 0 94 | 0 87<br>0 99 | 0 062 | 0 96 | 0 95<br>0 97 | 0 010 | 4 8 |
| 20 | ♀ | 75 | Chr pyelon | 1 0–1 5 | 5 3 | 3 | 4 2 | 7 60 | 4 06 | 1 84 | 1 36<br>2 30 | 0 470 | 0 99 | 0 80<br>1 21 | 0 [illegible]01 | 4 3 |
| 21 | ♂ | 64 | Chr glomer | 0 1 | 15 0 | 3 | 4 38 | 5 48 | 4 42 | 1 25 | 1 15<br>1 33 | 0 092 | 1 01 | 0 95<br>1 05 | 0 053 | 4 0 |
| 22 | ♀ | 45 | Polycysti k | 0 4–0 8 | 13 0 | 3 | 4 66 | 5 17 | 4 67 | 1 11 | 1 0<br>[illegible] | 0 101 | 1 00 | 0 98<br>1 04 | 0 030 | 5 2 |

Table II *Inulin clearance 5–15 ml/min/1 73 m*

| Pat no | Sex | Age | Diagnosis | Proteinuria (g/d) | Plasma-creat (mg/100 ml) | No of periods | Average plasma clearance | | | Clearance ratio | | | | | | Plasma alb (g/100 ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Creatinine inulin | | | Iothalamate inulin | | | |
| | | | | | | | Inulin | Creatinine | Iothalamate | Average | Range | S D | Averag | Range | S D | |
| 1 | ♀ | 80 | Nephroscler | 0–0 6 | 7 4 | 3 | 6 06 | 7 64 | 6 18 | 1 26 | 1 23<br>1 38 | 0 070 | 1 02 | 0 94<br>1 13 | 0 097 | 4 2 |
| 2 | ♂ | 19 | Chr glomer<br>Nephrotic | 5 5–7 5 | 8 0 | 3 | 6 16 | 10 92 | 6 29 | 1 78 | 1 60<br>1 95 | 0 176 | 1 03 | 0 94<br>1 11 | 0 085 | 2 0 |
| 3 | ♀ | 71 | Chr pyelon | 1 3 | 4 9 | 3 | 8 09 | 12 56 | 8 78 | 1 55 | 1 55<br>1 56 | 0 007 | 1 09 | 1 05<br>1 12 | 0 035 | 3 6 |
| 4 | ♀ | 24 | Chr glomer | 3 0–3 5 | 8 0 | 3 | 9 16 | 9 87 | 8 08 | 1 08 | 1 00<br>1 14 | 0 071 | 0 89 | 0 81<br>0 94 | 0 070 | 4 4 |
| 5 | ♂ | 37 | Chr glomer | 4 5–6 5 | 6 6 | 3 | 10 13 | 17 99 | 8 80 | 1 77 | 1 50<br>2 29 | 0 450 | 0 99 | 0 70<br>0 88 | 0 160 | 3 5 |
| 6 | ♂ | 19 | Chr glomer<br>Nephrotic | 12–15 | 6 0 | 3 | 10 15 | 15 56 | 10 50 | 1 53 | 1 49<br>1 62 | 0 075 | 1 10 | 1 00<br>1 04 | 0 043 | 3 3 |
| 7 | ♀ | 35 | Chr pyelon | 0 7–0 9 | 5 2 | 2 | 10 23 | 18 21 | 10 43 | 1 78 | 1 77<br>1 79 | 0 014 | 1 05 | 1 00<br>1 03 | 0 036 | 4 2 |
| 8 | ♂ | 37 | Chr glomer | 2–3 | 5 5 | 3 | 10 70 | 16 58 | 10 66 | 1 55 | 1 43<br>1 64 | 0 107 | 1 02 | 0 94<br>0 99 | 0 039 | 4 2 |
| 9 | ♀ | 45 | Chr pyelon | 1 2 | 4 9 | 3 | 10 72 | 13 27 | 10 27 | 1 24 | 1 22<br>1 28 | 0 035 | 0 96 | 0 93<br>1 01 | 0 042 | 4 1 |
| 10 | ♀ | 37 | Chr glomer | 2 1–3 3 | 5 0 | 3 | 11 09 | 16 37 | 11 55 | 1 48 | 1 32<br>1 64 | 0 160 | 1 04 | 1 01<br>1 06 | 0 029 | 3 8 |
| 11 | ♀ | 55 | Chr pyelon | 0 6–1 2 | 3 9 | 3 | 11 39 | 17 75 | 10 69 | 1 55 | 1 45<br>1 66 | 0 105 | 0 94 | 0 91<br>0 96 | 0 027 | 4 3 |
| 12 | ♂ | 42 | Nephroscler | 3–4 | 7 4 | 3 | 11 55 | 12 72 | 10 29 | 1 10 | 1 05<br>1 14 | 0 046 | 0 89 | 0 87<br>0 93 | 0 032 | 4 1 |
| 13 | ♀ | 44 | Chr pyelon | 0 7 2 1 | 4 0 | 3 | 14 83 | 31 98 | 18 54 | 2 12 | 1 79<br>2 74 | 0 537 | 1 24 | 1 21<br>1 31 | 0 047 | 3 8 |

Table III *Inulin clearance 15–25 ml/min/1 73 m²*

| Pat no | Sex | Age | Diagnosis | Proteinuria (g/d) | Plasma-creat (mg/100 ml) | No of periods | Average plasma clearance: Inulin | Average plasma clearance: Creatinine | Average plasma clearance: Iothalamate | Clearance ratio, Creatinine inulin: Average | Creatinine inulin: Range | Creatinine inulin: S D | Iothalamate inulin: Average | Iothalamate inulin: Range | Iothalamate inulin: S D | Plasma alb (g/100 ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ♂ | 54 | Chr glomer | 2–3 | 3 5 | 3 | 15 58 | 25 16 | 13 78 | 1 63 | 1 59<br>1 67 | 0 040 | 0 88 | 0 85<br>0 91 | 0 031 | 2 6 |
| 2 | ♀ | 59 | Chr pyelon | 0–0 5 | 2 3 | 3 | 16 18 | 19 34 | 14 03 | 1 23 | 0 74<br>1 98 | 0 660 | 0 87 | 0 83<br>0 90 | 0 035 | 3 3 |
| 3 | ♀ | 38 | Chr pyelon | 2 3 | 4 0 | 3 | 18 35 | 22 65 | 17 33 | 1 23 | 1 20<br>1 23 | 0 031 | 0 95 | 0 93<br>0 97 | 0 016 | 4 3 |
| 4 | ♂ | 44 | Chr glomer<br>Nephrotic | 15–20 | 3 3 | 3 | 20 21 | 31 85 | 17 51 | 1 59 | 1 47<br>1 69 | 0 110 | 0 88 | 0 70<br>1 11 | 0 210 | 0 9 |
| 5 | ♂ | 56 | Nephroscler | 2–3 | 2 9 | 3 | 21 84 | 24 64 | 19 39 | 1 13 | 1 08<br>1 18 | 0 050 | 0 89 | 0 86<br>0 91 | 0 026 | 3 6 |
| 6 | ♀ | 65 | Chr pyelon | 3–4 | 2 9 | 3 | 22 30 | 23 05 | 20 84 | 1 04 | 1 00<br>1 08 | 0 041 | 0 94 | 0 85<br>1 00 | 0 115 | 3 5 |
| 7 | ♀ | 47 | Chr glomer<br>Nephrotic | 20–25 | 2 0 | 3 | 22 77 | 60 55 | 20 02 | 2 66 | 2 35<br>3 08 | 0 375 | 0 89 | 0 77<br>1 00 | 0 071 | 0 8 |
| 8 | | 49 | Chr pyelon | 0–0 8 | 1 6 | 3 | 24 95 | 42 06 | 25 09 | 1 69 | 1 52<br>1 81 | 0 150 | 1 01 | 0 97<br>1 08 | 0 061 | 3 3 |

reduced renal function i e inulin clearance <25 ml/min The object of the present study has been to investigate this group of patients using simultaneous inulin creatinine and [125]Iothalamate clearance

## MATERIAL

Forty three patients 18 men and 25 women were studied. Renal function as measured by serum creatinine varied from 1 6 to 29 5 mg/100 ml Patients were divided into three groups on the basis of the inulin clearance Group I included 22 patients aged 14 to 75 years, with an inulin clearance <5 ml/min. Five were oliguric none were clinically overhydrated In group II were 13 patients, aged 19 to 80 years with an inulin clearance between 5 and 15 ml/min Group III consisted of eight patients, 38 to 65 years with an inulin clearance between 15 and 25 ml/min Other relevant data e g diagnoses, age sex, are given in Tables I II and III

## METHODS

Because of the reduction in renal function it was necessary in several cases to modify the classic clearance technique (2 16 45) Oral fluid intake was initiated two hours before the beginning of the clearance period Thirty-eight patients who had retained the ability to excrete water drank 300–500 ml/h the other five were given 50–100 ml/h Blood and urine samples for determination of blind values were taken half an hour before the start of the clearance period In all patients with the exception of two three clearance periods were measured. Voiding took place in all patients by spontaneous bladder emptying at the bedside A urine volume of at least 100 ml was aimed at This necessitated long clearance periods—from 24 to 170 min A constant infusion technique using a Holter infusion pump was used The equilibrium time ranged from 30 to 80 min Blood samples were taken 6 min before the midpoint of each clearance period (7)

The blood glucose level was determined before and after each study

### *Inulin*

The priming and sustaining doses were given so that the plasma concentration remained between 15 and 25 mg/100 ml Heyrovsky's method (22) was used for analysis. This method involves the photometric determination of a colour reaction between betaindolacetic acid and fructose in concentrated HCl Proteinuria does not interfere with the analysis and the interference of glucose is negligible Duplicate determinations were made in all analyses

### *Creatinine*

The autoanalyser method was used (Technicon 11 b) (49) with slight modifications in order to increase sensitivity

*Iothalamate*

Sodium iothalamate tagged with ¹²⁵I was used in all clearance studies Twenty to 25 $\mu c$ iothalamate was added to the total amount of inulin needed for the measurement of clearance The mixture was then divided into priming and sustaining doses so that a plasma activity of 800 to 1 800 counts/min/ml was obtained Activity was measured in a gamma-counter equipped with a dual-channel pulse height analyser to a statistical inaccuracy of less than 1%

*Clearance*

Clearance was calculated according to the formula $C = U \times V/P \times 173/SA$ where $C$ is clearance in ml/min $U$ the concentration in the urine in mg/100 ml or counts/min/ml, $V$ the urine volume in ml/min $P$ the concentration in the plasma in mg/100 ml or counts/min/ml $SA$ the patient s surface area in m according to Dubois s nomogram for height and weight

## RESULTS

*Group I* (inulin clearance < 5 ml/min)

Twenty two patients 12 men and 10 women Serum creatinine varied between 5 3 and 29 5 mg/100 ml Sixty five clearance studies were done The mean value for inulin clearance was 2 70 ml/min with a coefficient of variation of 17 55 % for creatinine clearance 3 43 ml/min with a coefficient of variation of 19 24 % and for iothalamate clearance 2 63 ml/min with a coefficient of variation of 17 00 %

The ratio creatinine/inulin clearance ($^{C}Cr/^{C}In$) varied from 0 94 to 1 84 with a mean of 1 27 ± 0 135 The ratio of iothalamate/inulin clearance ($^{C}Io/^{C}In$) varied from 0 85 to 1 14 with a mean of 0 98 ± 0 060

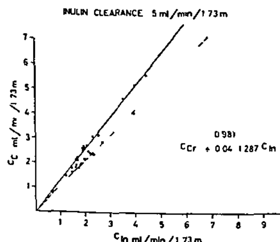



*Fig 1* Inulin clearance compared to endogenous creatinine clearance showing the fitted regression line (solid line) and the theoretical ratio of 1 0 (interrupted line)

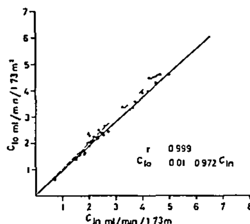



*Fig* Inulin clearance compared to iothalamate clearance showing the fitted regression line (solid line) and the theoretical ratio of 1 0 (interrupted line)

$^{C}Cr/^{C}In$ showed no correlation with the magnitude of proteinuria Neither was there any association between the concentration of serum albumin and the abovementioned ratio The largest $^{C}Cr/^{C}In$ ratio was seen in patient no 20 with chronic pyelonephritis and an exceptionally low serum creatinine for the group There was no relation ship between the nature of the renal disease and the size of $^{C}Cr/^{C}In$ The values for inulin creatinine and iothalamate clearance are given in Table I and Figs 1 and 2 in which the regression equation and $r$ value are shown As can be seen creatinine clearance overestimated the G F R in 21 patients The difference is significant $p < 0.05$ Iothalamate clearance was a little below inulin clearance but the difference is not significant The large coefficients of variation may be accounted for by the small urine volumes—from 0 31 to 2 70 ml/min—associated with spontaneous voiding

*Group II* ($^{C}In$ 5–15 ml/min)

There were 13 patients in this group five men and eight women Serum creatinine values ranged from 3 9 to 8 0 mg/100 ml In 38 clearance studies a mean value was found for $^{C}In$ of 10 02 ml/min with a coefficient of variation of 6 95 for $^{C}Cr$ of 15 49 ml/min with a coefficient of variation

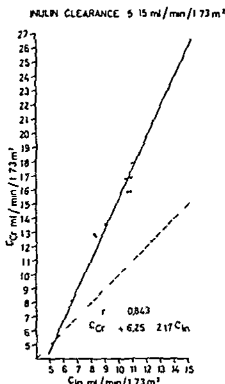



*Fig 3* Inulin clearance compared to endogenous creatinine clearance showing the fitted regression line (solid) and the theoretical ratio of 1 0 (interrupted line)

of 11 17% and for ᶜIo of 10 07 ml/min with a coefficient of variation of 6 84%

ᶜCr/ᶜIn varied from 1 08 to 2 12 with a mean of 1 52 ± 0 143 ᶜIo/ᶜIn ranged from 0 89 to 1 24 with a mean of 1 00 + 0 057 The ᶜCr/ᶜIn in this group was clearly higher than in group I Proteinuria in group II averaged 3 4 g/d contrasted with 2 2 g/d in group I

The relationship between inulin creatinine and iothalamate clearance is given in Table II and Figs. 3 and 4 in which the regression equation and *r* value are shown As can be seen, creatinine clearance was again greater than G F R. ($p < 0 001$) There was no difference between inulin and iothalamate clearance for the group as a whole The coefficients of variation were all within the normal limits of variation Urine volumes in this group were between 1 76 and 6 26 ml/min

*Group III* (ᶜIn 15–25 ml/min)

This group included eight patients one man and seven women. Serum creatinine ranged from 1 6 to 4 0 mg/100 ml In 24 clearance studies the mean values for the group were as follows ᶜIn 20 27 ml/min (coefficient of variation 8 32%) ᶜCr 31 16 ml/min (coefficient of variation 4 13%) and ᶜIo 18 64 ml/min (coefficient of variation 8 97%)

ᶜCr/ᶜIn varied from 0 74 to 3 08 with a mean of 1 53 ± 0 182 ᶜIo/ᶜIn ranged from 0 77 to 1 11 with a mean of 0 92 ± 0 071

ᶜCr/ᶜIn was of the same magnitude as in group II even though mean proteinuria amounted to 6 6 g/d in this group The largest ratios were seen in patients with the nephrotic syndrome but no correlation could be found between the level of proteinuria the concentration of serum albumin and the magnitude of ᶜCr/ᶜIn

ᶜIo/ᶜIn was independent of proteinuria The smallest ratios were seen in patients with the greatest reduction in serum albumin

The relationship between inulin creatinine and iothalamate clearance is shown in Table III and Figs 5 and 6 in which the regression equation and the *r* value are given

ᶜCr overestimated the G F R markedly ($p < 0 01$) There was a slight variance between iothalamate and inulin clearance ($p = 0 25$) The coefficients of variation are within normal limits Urine volume per min amounted to 0 74 to 12 43 ml

## DISCUSSION

The elementary importance of accurate urine collection during clearance studies has been stressed

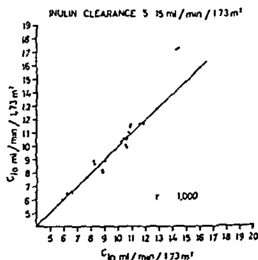



*Fig 4* Inulin clearance compared to iothalamate clearance, showing identity

since the introduction of this procedure into clini cal medicine (16 23 46) This has resulted in the employment of catheters bladder washout and suprapubic expression of urine A few investiga tors have lately however used spontaneous blad der emptying in healthy individuals and those with only a slight reduction of kidney function Instru mentation of the urinary tract in the performance of clearance studies in patients with severely re duced renal function is hazardous because of the risk of infection This applies particularly to pa tients in terminal uraemia when transplantation is a possibility

Complete bladder emptying can be achieved in these patients with adequate water loading, careful instruction and supervision encouragement and patience An objective measure of the success of these efforts is afforded by calculation of the coef ficient of variation The size of this coefficient without catheterization for inulin clearance is stated by Healy (21) to be 6 4% by Mandel and Jones (26) to be 8 5–17% and by Goldring and Chasis (45) to be 8 7–17 8 Values between 8 1 and 17 8% are given for creatinine clearance (26)

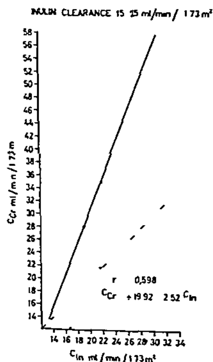



*Fig 5* Inulin clearance compared to endogenous creatinine clearance showing the fitted regression line (solid line) and the theoretical ratio of 1 0 (interrupted line)

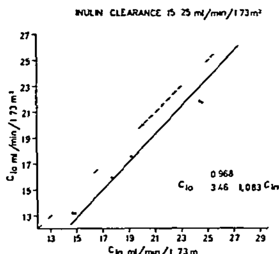



*Fig 6* Inulin clearance compared to iothalamate clearance, showing the fitted regression line (solid line) and the theoretical ratio of 1 0 (interrupted line)

In group I the coefficients of variation were thus near the upper limits of normal" Factors contributing to this were the deadspace effect de scribed by Sarre (39) in connection with small urinary volumes together with the influence even small amounts of residual urine have on the total urinary volume However since the clearance periods were quite long and since the coefficients are mutually of the same order of magnitude the clearance values obtained without catheterization as here described may be accepted as representa tive for G F R The coefficients of variation in the other two groups indicate that supervision of patients and the techniques of analysis were satis factory

Since the introduction of endogenous creatinine clearance as a measure of glomerular filtration rate there has been a great deal of discussion about the value of the method and its physiological im portance In patients with normal renal function the method appears to be a reliable expression of glomerular filtration, as all investigators report a creatinine/inulin clearance ratio around 1 0 Marked variations in these clearance ratios are however seen Thus Steinitz and Turkand (48) found ratios varying from 0 73 to 1 17 (1 03) Miller and Winkler (30) 0 8 to 1 5 (1 07) and Smith et al (44) 0 81 to 1 08 (0 94)

With renal insufficiency however clearance ratios greater than one are always found The size of the ratio has been variously reported. Brod

and Sirota (5) using a modified Folin Wu technique could not demonstrate a significant difference between patients with normal renal function and renal insufficiency and reported a mean ratio of 1 04 Using the same method Doering (9) found in 1953 in patients with different degrees of renal insufficiency a ratio of 1 13 and using Popper et al s method (36) a ratio of 1 33 Miller and Winkler (30) and Steinitz and Turkand (48) report 1 38 and 1 37 respectively Berlyne (3) found in 1964 a ratio of 1 85 in nephrotic patients with a slight reduction in renal function

An essential cause of the divergent findings as regards the size of the ratio is the technique used for analysis of chromogenic material (34) In patients with normal kidney function 80 % of the total creatinine chromogen is made up of creatinine (32) but in azotaemic patients the ratio between non-creatinine chromogenic material and creatinine is much larger (29) In patients with a serum creatinine above 20 mg/100 ml however non-creatinine chromogenic material makes up only about 5 % (10) and the amount of pseudo-creatinine" present bears no constant relationship the actual serum creatinine (21) When the method is used the degree to which non-creatinine chromogenic material is dialysable also plays a role Healy s studies (21) of simultaneous creatinine and inulin clearance have however shown that regardless of whether creatinine total chromogen or creatinine measured with the autoanalyser method is used G F R is overestimated in renal insufficient patients

Provided that inulin clearance also in renal insufficient patients gives a true expression of the glomerular filtration then the large ratios must reflect a changed pattern of excretion for endogenous creatinine in these patients. Proteinuria appears to increase creatinine excretion Miller et al (32) Brod and Sirota (5) and Berlyne (3) found large ratios from 1 38 to 1 85 in patients with the nephrotic syndrome To what degree there is a tubular secretion of creatinine in renal insufficient patients with proteinuria is as yet unclear (5 6, 27 44) Brod and Sirota demonstrated in their patient that a large $^{C}Cr/^{C}In$ ratio could be reduced to 1 0 using caronamide Mandel et al (27) were not however able to suppress the ratio with the use of benemid

The ratio of 1 27 in group I is probably due to the fact that non-creatinine chromogenic material makes up a smaller fraction of the total creatinine when the serum creatinine is markedly elevated.

If endogenous creatinine is secreted by the tubulus in renal insufficiency the lower ratios in this group may also be explained as due to the phenomenon of self-depression

In groups II and III identical mean ratios for $^{C}Cr/^{C}In$ were found even though the serum creatinine values in group II amounted to 3 9–8 0 mg/100 ml and in group III 1 6–4 0 mg/100 ml. The explanation might be sought in differences in the degree of proteinuria It is however evident from the data presented that the ratio does not demonstrate any constant or precise relationship to the degree of proteinuria Neither could any certain association be shown between reduction of the serum albumin and the size of the ratio

Iothalamate fulfils the criteria that Smith et al. (46) have formulated for a substance used for determining G F R Quantitative estimation is rapid and accurate The substance is physiologically inactive Excretion is independent of the plasma concentration (42) Renal excretion does not take place in aglomerular fish and stop-flow studies in dogs show that a parallel exists between inulin and iothalamate concentrations in the tubular fluid (17) Clinical studies in individuals with an inulin clearance between 15 and 145 ml/min have demonstrated agreement between the two substances, with a mean ratio of 1 00 (13)

In the present study a mean ratio of 0 97 for $^{C}Io/^{C}In$ was found for the group as a whole It is also evident that there is good agreement between inulin and iothalamate clearance in cases where the inulin clearance is less than 15 ml/min as the mean ratio for group I was 0 98 and for group II 1 00 This means that the excretion of iothalamate is independent of proteinuria and the concentration of serum albumin In addition, the standard deviation of the mean ratio amounted to no more than 0 060 It appears that iothalamate provides a new standard reference substance for measuring G F R.

## REFERENCES

1 Addis, T., Barett, E., Poo L. J Urken, H J & Lippmann, H W The relation between protein consumption and diurnal variations of the endogenous creatinine clearance in normal individuals. J clin. Invest. 30 -06, 1951
2. Balint, P., Nierenclearance Fischer Jena 1965

3 Berlyne G M Varley H Nilwarangkur S & Hoernig M Endogenous-creatinine clearance and glomerular filtration rate Lancet 2 *874* *1964*
4 Borsness R & Taussky H H On the colorimetric determination of creatinine by the Jaffe reaction J biol Chem 158 581 1945
5 Brod, *J* & Sirota *J H* The renal clearance of endogenous creatinine in man J clin Invest 27 645 1948
6 Bucht, H On the tubular excretion of thiosulphate and creatinine under the influence of caronamide Scand. J clin Lab Invest. 1 270 1949
7 Brun C Hilden, T & Raaschou F The significance *of the difference in systemic arterial and venous plasma* concentrations in renal clearance methods J clin Invest 28 144 1949
8 Concannon, J P Summers R. E Brewer R Cole C Weil C & Foster W D $I^{125}$ allyl inulin for the determination of glomerular filtration rate Amer J Roentgenol 92 302, 1964
9 Doering, P Koch R Schroeter H & Schwab M Die endogene Kreatininclearan e beim nierenkranken Menschen Klin Wschr 31/22 489 1953
10 Doolan P O Alpen E L & Theil G B A clinical appraisal of the plasma concentration and endogenous clearance of creatinine Amer J Med 32 65 1962
11 Edwards K D G & Whyte H M Plasma creatinine level and creatinine clearance as tests of renal function Aust. Ann Med 8 218 1959 *b*
12 Effersøe P Relationship between endogenous 24 *hour creatinine clearan e and serum creatinine concen*tration in patients with chronic renal disease Acta med scand 156 429 1957
13 *Elwood* C M & Sigmann E M The measurement of glomerular filtration rate and effective renal plasma flow in man by iothalamate $^{125}$I and iodopyracate I Circulation 36 441 1967
14 Elwood C M Sigmann E M & Treger C The measurement of glomerular filtration rate with $^{125}$I sodium iothalamate (Conray) Brit J Radiol 40 581 1967
15 Ferguson, M H Olbrich O Robson J S & Stewart C P The use of inulin clearance as a measure of glomerular filtration. Quar J exp Physiol 35 251 1950
16 Goldring, W & Chasis H Hypertension and hypertens e disease The Commonwealth Fund New York 1944
17 Griep R. J & Nelp W B Mechanism of renal excretion of iodine $^{131}$-sodium iothalamate (Conray) J nuchl Med 7 60 1966
18 Haugen, H N & Blegen, E M The true endogenous creatinine clearance Scand J clin Lab Invest 5 *67* *1953*
19 — Plasma creatinine *concentration and creatinine* clearance in clinical work Ann intern Med 43 731 1955
20 Healy J K Edwards K D G & Whyte H M Simple tests of renal function using creatinine phenol sulphonphthalein and pitressin J clin. Path 17 557 1964
21 Healy J K Clinical assessment of glomerular filtration rate by different forms of creatinine clearance and a modified urinary phenolsulphonphthalein excretion test. *Amer J Med 44 348 1968*
22 Heyrovsky A A new method for the determination of inulin in plasma and urine Clin chim. Acta 1 470 1956
23 Josephson B & Godin A Om bestamning av glomerulusfiltrationen Nord Med 18 893 1943
24 Kennedy T J Jr & Kleh J The relationship between the clearance and the plasma concentration of inulin in normal man J clin. Invest 32 90 1953
25 Lubowitz, H Slatopolsky E Shankel S Rieselbach R. E & Bricker N S Glomerular filtration rate *J Amer med. Ass* 199 *100* *1967*
26 Mandel E E & Jones F L Studies in nonprotein nitrogen III Evaluation of methods measuring creatinine J Lab clin Med 41 323 1953
27 Mandel E E Jones F Willis M J & Cargill W H Renal excretion of creatinine and inulin in man. J Lab clin Med 42 621 1953
28 Marsh D & Frazier C Reliability of inulin for *determining volume flow in* rat renal cortical tubules Amer J Physiol 209 283 1965
29 Miller B F & Dubos, R J Studies on the presence *of creatinine in human blood. J biol Chem. 121 447* 1937
30 Miller B F & Winkler A N The renal excretion of endogenous creatinine in man Comparison with exogenous creatinine and inulin J clin Invest 17 31 1938
31 Miller J., Alving A S & Rubin, J The renal excretion of inulin at low plasma concentrations of this compound and its relationship to the glomerular filtration rate in normal nephrotic and hypertensive individuals J clin Invest 19 89 1940
32 *Miller B F Leaf A L Mamby A R & Miller Z* Endogenous creatinine clearance as a measure of glomerular filtration rate in the diseased human kidney *J* clin. Invest. *31 309 1952*
33 Nelp W B Wagner H M Jr & Reba R C Renal excretion of vitamin B and its use in measurement of glomerular filtration rate in man J Lab clin Med 63 480 1964
34 Owen J A Iggo B Scandrett F J & Stewart, C P The determination of creatinine in plasma or serum and in urine a critical examination Biochem J 58 4 6 1954
35 Popper H & Mandel E E Filtrations und Resorptionsleistung in der Nierenpathologie Ergebn. inn Med. Kinderheilk 53 685 1937
36 Popper H Mandel E & Mayer H Zur Kreatininbestimmung im Blute Biochem Z 291 354 1937
37 *Richards* A N *Bott, P A & Westfall* B B Renal excretion of inulin creatinine and xylose in normal dogs Proc Soc exp Biol 32 73 1934
38 *Rehberg P B Studies on kidney function* I The ratio of filtration and reabsorption in the human kidney Biochem J 0 447 19 6
39 Sarre H Nierenkrankheiten *Thieme Stuttgart 1959*
40 Schirmeister J K. Willaenn H Kiefer H & Hallauer W Fur und wider die Brauchbarkeit der en

dogenen Kreatininclearance in der funktionellen Nierendiagnostik. Dtsch med Wschr 35 1640 1964
41 Shannon J A. & Smith H W The excretion of inulin xylose and urea by normal and phlorizinized man J clin Invest. 14 393 1935
42. Sigmann E M Elwood, C Reagan, M E Morris, A M & Catanzaro A. The renal clearance of I[125] labelled sodium iothalamate in man Invest. Urol 2 437 1960
43 Sigmann E M Elwood C & Knox, F Measurement of glomerular filtration rate in man with sodium iothalamate I (Conray) J nucl. Med 7 60 1966
44 Smith W W., Finkelstein N & Smith H W Renal excretion of hexitols (sorbitol mannitol and dulcitol) and their derivatives (sorbitan isomannide and sorbide) and of endogenous creatinine like chromogen in dog and man J biol Chem. 135 231 1940
45 Smith H W Goldring, W & Chasis H The measurement of the tubular excretory mass, effective *blood flow and filtration rate in the normal kidney* J clin Invest 17 263 1938
46 Smith H W The kidney Oxford University Press New York 1951
47 — Principles of renal physiology Oxford University Press, New York 1956
48 Steinitz, K. & Turkand H The determination of the glomerular filtration by the endogenous creatinine clearance J clin Invest. 19 285 1940
Technicon method Autoanalyzer N methodology Chauncey New York 1963
alser M Davidson, D C & Orloff J The renal clearance of alkali-stable inulin J clin Invest 34 1520 1955

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# THROMBOCYTOPENIA IN HEART FAILURE

## PRELIMINARY REPORT

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A recent report on the frequent occurrence of asymptomatic thrombocytopenia in heart patients *during treatment with thiazide diuretics* (5) prompted us to observe a similar series of patients in a prospective study This is a preliminary report of our results indicating that the low platelet counts observed are for the most part due to congestion rather than drug toxicity

## MATERIAL

The *series consisted of 78 consecutively admitted patients* with congestive heart failure taking digoxin (24 patients) or digoxin and thiazide diuretics (32 patients) The diuretic used was furosemide in almost every case the period varying from 2 weeks to 3 years. The patients receiving other treatment such as spironolactone ethacrynic acid or quinidine were taken as a third group (22 patients) but patients having other medications or diseases which are known causes of thrombocytopenia were excluded. A fourth group consisted of 16 ambulatory well-compensated heart patients taking digoxin and thiazides. Platelets were counted on two different days, and the mean of the results was recorded

## RESULTS

The platelet counts *from the patients are seen in* Table I The hospitalized patients taking digoxin or digoxin and diuretics had low platelet counts of the same order viz. 37 and 38 Of the third group of patients taking additional drugs most (64% ) *had low* platelet counts in contrast with the ambulatory patients who all had normal thrombocytes During treatment we recorded an increase in the platelet count on nine occasions and decrease on five None of the patients had any bleeding tendency

## DISCUSSION

In their series of patients treated with digoxin and chlorthalidon Kutti and Weinfeld (5) found platelet counts below 100 000/mm³ in 26 % of their patients whereas we could find similar counts only in 6 % of our patients who were using for the most part a combination of digoxin and furosemide Moreover all our well balanced outpatients had normal platelet counts on the same treatment The discrepancy in the results may be explained in two ways 1) the drugs used have different *toxicity for platelets* or 2) *the low platelet* counts in most cases are not related to the drug but rather to the disease itself

If the drug had been the cause of the low platelet counts recorded one would have expected their further decrease during treatment with the same drug but this did not happen In contrast the lowest counts were recorded in patients with poorest heart compensation The development of

Table I *Platelet counts in the various groups of patients*

| Treatment | No of pats | Platelet count per mm < 100 000 (n) | ( ) | 100 000–150 000 (n) | ( ) | > 150 000 (n) | ( ) |
|---|---|---|---|---|---|---|---|
| Digoxin | 24 | — | — | 9 | 37 | 15 | 63 |
| Digoxin and thiazides | 32 | 3 | 10 | 9 | 28 | 0 | 62 |
| Digoxin thiazides additional drugs | 22 | 3 | 14 | 11 | 50 | 8 | 36 |
| Digoxin and thiazides ambulatory | 16 | — | — | — | — | 16 | 100 |
| Total | 94 | 6 | 6 | 29 | 31 | 59 | 63 |

the platelet count seemed to be related to the changes in the clinical condition not to the treatment Even in the series published by Kutti and Weinfeld (5) most patients with thrombocytopenia had large heart volumes as an indication of decompensation

Congestive splenomegaly is a well known cause of thrombocytopenia (2) the main mechanism being a pooling of platelets in the spleen (1) The congestion of the organs of the upper abdomen in heart failure includes the spleen (3) One would think that the low platelet counts in patients with congestive heart failure would thus be caused by an excessive pooling of the platelets in the spleen Our preliminary results with $^{51}Cr$ labelled platelets indicate a normal or slightly shortened survival and high pooling of platelets similarly to the patients with splenomegaly (4) thus supporting our clinical assumption

## REFERENCES

1 De Gabriele G & Penington, D G Brit. J Haemat. 13 384 1967
2. Doan, C E. & Hall O Blood 1 10 1946.
3 Fowler N O Ann. intern. Med. 27 733 1947
4 Koulainen M Scand. J Haemat. Suppl. 5 1969
5 Kutti J & Weinfeld, A Acta med scand 183 245 1968

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# MALABSORPTION OF VITAMIN $B_1$ DURING TREATMENT WITH SLOW RELEASE POTASSIUM CHLORIDE

PRELIMINARY REPORT

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Megaloblastic anaemia in a patient with congestive heart failure and without any known reason for vitamin $B_1$ malabsorption aroused our interest in the possible relationship between the slow release potassium preparations and the absorption of vitamin $B_{12}$ This is a preliminary report of the low Schilling test values observed during treatment with slow release potassium chloride

## MATERIAL

The series consisted of 30 hospitalized patients given potassium supplementation. The preparation used was Kaliduron® (Orion Ltd Helsinki Finland) containing 500 mg of potassium chloride in a slow release tablet. The Schilling tests (5) were performed using 0 5 μg of $CoB_{12}$ (Institutt for Atomenergi Kjeller Norway) as a test dose Patients with pernicious anaemia or other known reasons for vitamin $B_{12}$ malabsorption were excluded.

## RESULTS

In 11 of the 30 patients studied the Schilling test value was lower during potassium treatment than without it The test values for these patients are presented in Table I The impairment of $B_1$ absorption during potassium treatment was striking in some instances the Schilling test value being on average 4 8 % compared with 11 4 % for the same patients without treatment In the remaining 19 patients the test values were normal with and without potassium the mean values being 15 4 % and 15 5 %

## DISCUSSION

The tendency to hypopotassaemia during treatment with diuretics has been the main reason for the need for long term potassium supplementation To relieve the hypochloraemic alkalosis occurring at the same time potassium chloride as a slow release preparation has been recommended (3) and widely used On the other hand absorption of vitamin $B_1$ occurs in the distal ileum (2) The intrinsic factor effect in the uptake of $B_1$ by ileal mucosa is optimal at pH 6 6 being minimal or absent at pH 5 5 and absent below that level (1) Thus it seems reasonable to think that in some patients the slow release potassium chloride tablets are able to cause an acidification of the contents of the ileum sufficient to disturb $B_{12}$ absorption Using radiotelemetric equipment (4) we have obtained measurements of pH in the intestines of some patients and the first observations support this view If the pH recorded during potassium chloride treatment was below 6 an impairment of the $B_1$ absorption was seen in the

Table I *Eleven patients who had low Schilling test values during treatment with slow release potassium*

| Pat no | Schilling test value | |
|---|---|---|
| | During potassium | Without potassium |
| 1 | 1 0 | 5 5 |
| 2 | 3 0 | 6 7 |
| 3 | 3 0 | 12 0 |
| 4 | 3 5 | 18 0 |
| 5 | 5 0 | 8 0 |
| 6 | 5 0 | 18 0 |
| 7 | 5 5 | 8 5 |
| 8 | 6 8 | 10 0 |
| 9 | 7 6 | 10 0 |
| 10 | 8 0 | 14 0 |
| 11 | 8 3 | 15 0 |
| Mean | 4 8 | 11 4 |

Table I *Survey population*

| Age group | 18-24 | 25 34 | 35-44 | 45-54 | 55-64 | Total |
|---|---|---|---|---|---|---|
| Total population | 88 | ‹96 | 81 | 179 | 109 | 953 |
| Did not attend | 11 | 13 | 9 | 6 | 9 | 48 |
| Attended | 77 | ‹83 | 272 | 173 | 100 | 905 |
| Per cent of total | 88 | 96 | 97 | 97 | 92 | 95 |

factories there is pollution from fumes, which may damage the vegetation Snow covers the ground by October November and usually melts during April

The field survey was conduct d between November 1966 and February 1967

## Population Studied

The male workers of a pulp mill of a large woodworking company were selected for study Apart from wood pulp the mill produces chlorine which is needed during the bleaching stages of wood pulp and various by products of cellulose The whole fa tory area is contaminated by the smell from various mercaptans re eased during the process. The workers do not come into contact with any other fumes, the exception being the workers of the chlorine factory who may oc asionally be exposed to low chlorine concentrations.

The industrial workers (excluding clerical staff) numbered 953 men their age distribution and the total of the population studied appear in Table I Those attending the study numbered 905 (95 of total)

## Procedure

men were requested to present themselves, in groups of 10- 0 in rooms appointed for the purpose on the factory premises A questionnaire was completed for each man and height (without shoes) and weight (without coat) were recorded In addition each subject performed some simple lung function tests A che t film (70× 0 mm roentgenogram or normal 35 35 cm, thoracic roentgenogram) taken not more than three years before was available in each case In most of the cases the roentgenogram had been taken during the year immediately preced ng the study

The questionnaire was essentially the same as the one recommended by the Medical Research Council s Committee on the Aetiology of Chron c Bronchitis and previously used in a great number of epidemiological stud es (17) All the forms were filled in by the same assistant, and the instructions for the use of the questionnaire were adhered to as closely as possible The questionnaire has been appended to a previous study (10) and in all essential points the technique of investigation was the same

To evaluate dyspnoea, the subjects were classified into five grades of severity grade I signifying no dyspnoea at all. These grades correspond roughly to the groups described earlier by Fletcher (4)

The men were classified by smoking habits into the following categories

1 Non smokers this group includes those who had never smoked and also those who had smoked at most one cigarette daily for one year or less, or the corresponding amount of other types of tobacco

2. Ex-smokers these included subjects who had stopped smoking one month or more before the date of the interview and could not be recorded as non-smokers, if less than one month had passed since smoking, the subject was placed in the respective group of smokers.

3 Smokers all those not included in groups 1 and 2. These were subdivided according to type of tobacco smoked (cigarettes, cigars pipe) and also according to the amount smoked at the time of the interview One cigarette was taken to represent one gramme of tobacco. The amount smoked earlier was disregarded.

## Pulmonary Function Tests

The following indices of ventilatory function were determined the forced vital capacity (FVC) the one second forced expiratory volume ($FEV_1$) and the latter expressed as a percentage of the former (FEV % ) The measurements were made with the spirometer described by McKerrow and his co-workers (16) The spirometer is so calibrated that the above entities are obtained as BTPS (body temperature and pressure saturated with water vapour) values, in which terms they are here expressed Checks of the calibration were made before each session Each subject made five expirations The first two were excluded and the mean of the last three calculated this was the definitive FEV or FVC fr m which the FEV was obtained No nose cl p was used.

## Diagnostic Criteria

*Chronic bronchitis*

Production of phlegm on most days for at least three months a year during at least two consecutive years, unless expectoration was attributable to some local or specific lung disease or asthma had been diagnosed at hospital and not yet cured.

*Asthma or airways obstruction*

The subject was included in this category if the diagnosis of asthma had been made earlier at hospital and he said it had not been cured, or if the FEV was 60 or less of the forced vital capacity in a subject who could not be diagnosed as having chronic bronchitis.

The broad term chronic non-specific lung disease (CNSLD) has been used to cover the above disorders. It will be clear from the defin tions that the diagnostic categories are mutually exclusive

*Pulmonary tuberculosis*

The diagnosis was made on the usual cl nical radiological and bacteriological criteria data recorded at the tuberculosis di pensary and the case re ords of hospitalized cases were available This group included the cases of "inactive" or quiescent tuberculosis in which no bacteria had been found but the scarring in the lungs was moderately or far advanced. This diagnosis was omitted

Table II *Smoking habits by age*

| Age group<br>No of pats. | 18 24<br>76 | | 25–34<br>273 | | 35–44<br>237 | | 45–54<br>150 | | 55–64<br>76 | | Total<br>812 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No | | No | | No | | No | | No | | No | |
| Non-smokers | 16 | 21 | 65 | 24 | 48 | 20 | 19 | 13 | 12 | 16 | 160 | 20 |
| Ex-smokers | 10 | 13 | 41 | 15 | 50 | 21 | 48 | 32 | 23 | 30 | 172 | 21 |
| Smokers | 50 | 66 | 167 | 61 | 139 | 59 | 83 | 55 | 41 | 54 | 480 | 59 |
| Cigarettes | 46 | 61 | 133 | 49 | 112 | 48 | 71 | 47 | 37 | 49 | 399 | 49 |
| Cigars pipe | 4 | 5 | 34 | 12 | 27 | 11 | 12 | 8 | 4 | 5 | 81 | 10 |
| Amount smoked | | | | | | | | | | | | |
| 1 14 g day | 23 | 30 | 57 | 21 | 43 | 18 | 6 | 17 | 17 | 22 | 166 | 20 |
| 15– 4 g day | 23 | 30 | 86 | 32 | 73 | 31 | 44 | 29 | 20 | 6 | 246 | 30 |
| 25 g day | 4 | 5 | 24 | 9 | 23 | 10 | 13 | 9 | 4 | 5 | 68 | 8 |

in the case of calcified hilar lymph nodes and small calcified foci in the lung parenchyma, and also in most cases of apparently healed minimal pulmonary changes these scars had often been noted years ago

*Other respiratory diseases*

This group included all diseases and anomalies not listed above intrathoracic or of the bony thorax, insofar as they could be assumed to be of clinical significance The conventional diagnostic methods were used.

## *Statistical Methods*

Standard statistical methods were used throughout Regression equations for two variables have been derived as described by Linder (14)

## RESULTS

Of the 905 men studied 93 (10 %) fell into the diagnostic groups pulmonary tuberculosis and/or other respiratory disease In the main they were cases with extensive fibrotic changes due to previous tuberculosis or pronounced pleural adhesions These were excluded and the results refer only to the remaining 812 men

### *Smoking habits and anthropometric data*

The smoking habits of the subjects studied are presented in Table II and the mean heights and weights in the various smoking categories in Table III The number of smokers seems to decrease with age while the number of ex-smokers correspondingly increases Most of the smokers smoked cigarettes cigar and/or pipe smokers represent only 17 of the total number of smokers and only 68 men (14 % of all smokers) smoked 25 g or more daily

As regards height the various groups do not differ significantly but the non smokers and ex smokers were on an average heavier than the smokers When comparing the ages in the various smoking categories no differences can be detected except that the ex smokers were on average 4–5 years older than the subjects in the other groups

### *Respiratory symptoms*

The respiratory symptoms analysed by age and smoking habits appear in Tables IV–VII and Fig 1

The smokers coughed considerably more than the non smokers the difference is found to be highly significant Non smokers were seldom affected by cough and age as such does not seem

Table III *Mean height weight and age in different smoking groups*

| | Non smokers | Ex smokers | Smokers (g day) 1–14 | 15 4 | 25– | Total |
|---|---|---|---|---|---|---|
| Height (cm) | | | | | | |
| 18 24 | 175 6 | 175 6 | 173 0 | 175 0 | 179 1 | 174 9 |
| 25–34 | 172.2 | 171 3 | 173 0 | 172 2 | 173 0 | 172 3 |
| 35–44 | 171 0 | 170 6 | 169 8 | 169 7 | 172 0 | 1 0 4 |
| 45–54 | 166 3 | 170 6 | 169 2 | 169 0 | 171 4 | 169 1 |
| 55–64 | 166 7 | 167 5 | 165 5 | 169 3 | 168 0 | 167 4 |
| 18–64 | 171 0 | 170 6 | 171 0 | 170 9 | 172 4 | 171 0 |
| Weight (kg) | | | | | | |
| 18– 4 | 75 6 | 77 1 | 71 3 | 73 8 | 65 5 | 73 4 |
| 25–34 | 76 1 | 76 9 | 76 5 | 76 2 | 76 6 | 76 4 |
| 35–44 | 78 2 | 77 7 | 76 8 | 74 6 | 79 1 | 76 8 |
| 45–54 | 76 9 | 81 7 | 73 0 | 74 6 | 77 8 | 77 0 |
| 55–64 | 82 6 | 81 2 | 72 8 | 71 0 | 77 5 | 76 6 |
| 18–64 | 77 7 | 79 0 | 74 9 | 74 8 | 77 1 | 76 4 |
| Mean age (y ) | 36 1 | 41 3 | 36 8 | 37 7 | 37 7 | 37 9 |

Table IV *Prevalence of cough in various age and smoking groups*

| | Non-smokers | | Ex smokers | | Smokers (g day) 1-14 | | 15-24 | | 25- | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No | | No | % | No | % | No | | No | % | No | |
| 1 Cough in the morning, winter | | | | | | | | | | | | |
| 18- 4 | — | — | — | — | — | — | 3 | 13 | — | — | 3 | 4 |
| 25-34 | — | — | 1 | 2 | 11 | 19 | 15 | 17 | 8 | 33 | 35 | 13 |
| 35-44 | 1 | 2 | 1 | 2 | 6 | 14 | 22 | 30 | 9 | 39 | 39 | 16 |
| 45-54 | 2 | 11 | 3 | 6 | 5 | 19 | 17 | 39 | 7 | 54 | 34 | 23 |
| 55-64 | — | — | 1 | 4 | 6 | 35 | 5 | 25 | 3 | 75 | 15 | -0 |
| 18-64 | 3 | 2 | 6 | 3 | -8 | 17 | 62 | 25 | 27 | 40 | 1 6 | 16 |
| 2 Cough in the morning summer | | | | | | | | | | | | |
| 18-24 | — | — | — | — | — | — | 1 | 4 | — | — | 1 | 1 |
| 25-34 | — | — | — | — | 9 | 16 | 9 | 10 | 7 | 29 | 25 | 9 |
| 35-44 | 1 | 2 | 1 | 2 | 3 | 7 | 13 | 18 | 6 | -6 | 24 | 10 |
| 45-54 | 1 | 5 | 2 | 4 | 5 | 19 | 13 | 30 | 5 | 38 | -6 | 17 |
| 55-64 | — | — | — | — | 5 | 29 | 4 | -0 | 1 | 25 | 10 | 13 |
| 18-64 | 2 | 1 | 3 | 2 | 22 | 13 | 40 | 16 | 19 | 28 | 86 | 11 |
| 3 Cough all day winter | | | | | | | | | | | | |
| 18--4 | — | — | — | — | — | — | 1 | 4 | — | — | 1 | 1 |
| 25-34 | — | — | — | — | 6 | 11 | 8 | 9 | 7 | 29 | 21 | 8 |
| 35-44 | 1 | 2 | 1 | 2 | 3 | 7 | 13 | 18 | 4 | 17 | 22 | 9 |
| 45-54 | 2 | 11 | 3 | 6 | 4 | 15 | 9 | -0 | 4 | 31 | 22 | 15 |
| 55-64 | — | — | 1 | 4 | 4 | 24 | 1 | 5 | 2 | 50 | 8 | 11 |
| 18-64 | 3 | 2 | 5 | 3 | 17 | 10 | 32 | 13 | 17 | 25 | 74 | 9 |
| 4 Cough all day summer | | | | | | | | | | | | |
| 18- 4 | — | — | — | — | — | — | 1 | 4 | — | — | 1 | 1 |
| 25-34 | — | — | — | — | 5 | 9 | 3 | 3 | 6 | 25 | 14 | 5 |
| 35-44 | 1 | 2 | — | — | 1 | 2 | 7 | 10 | 2 | 9 | 11 | 5 |
| 45-54 | — | — | 2 | 4 | 4 | 15 | 6 | 14 | 2 | 15 | 14 | 9 |
| 55-64 | — | — | — | — | 3 | 18 | 1 | 5 | 1 | 25 | 5 | 7 |
| 18-64 | 1 | 1 | 2 | 1 | 13 | 8 | 18 | 7 | 11 | 16 | 45 | 6 |
| Cough 3 months in the year | | | | | | | | | | | | |
| 18 -4 | — | — | 2 | -0 | — | — | 4 | 17 | — | — | 6 | 8 |
| -5-34 | 2 | 3 | 1 | - | 15 | 6 | 24 | -8 | 9 | 38 | 51 | 19 |
| 35-44 | 2 | 4 | 1 | 2 | 9 | -1 | -6 | 36 | 10 | 43 | 48 | 0 |
| 45-54 | 2 | 11 | 6 | 12 | 9 | 35 | 20 | 45 | 8 | 62 | 45 | 30 |
| 55-64 | — | — | 3 | 4 | 7 | 41 | 11 | 55 | 4 | 100 | -3 | 30 |
| 18 24 | 6 | 4 | 11 | 6 | 40 | 24 | 85 | 35 | 31 | 46 | 173 | 1 |

to play any part in the prevalence of cough The same applies to phlegm production it was of much greater amount and frequency among smokers than among non-smokers or ex-smokers No age trend appears particularly not when studying the groups of non smokers and ex smokers It may be noticed that phlegm production was more frequent compared with coughing

It is evident and understandable that dyspnoea increases with advancing age Non smokers do not differ from smokers as distinctly as they do in the case of cough and phlegm production yet by the chi square test—taking all smokers as one group—the difference in prevalence of dyspnoea between non smokers and smokers can be shown to be significant ($P < 0\,01$)

Wheezing is more common among smokers than among non smokers ($P < 0\,001$) but age is not found to affect the frequency of this symptom The effect of weather increases with age ($P < 0\,001$) whereas the part played by smoking is not demonstrable The prevalence of rhinitis does not differ from one age group to another nor is it different in the various smoking categories While respiratory diseases are more frequent among smokers than non smokers ($P < 0\,001$) age does not affect their prevalence

Table VIII illustrates the prevalence of CNSLD

Table V *Prevalence of phlegm production in various age and smoking groups*

| | Non smokers | | Ex smokers | | Smokers (g/day) 1–14 | | 15–24 | | 25– | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No | | No | | No | | No | | No | | No | |
| 1 Phlegm in the morning winter | | | | | | | | | | | | |
| 18–24 | — | — | 1 | 10 | — | — | 3 | 13 | — | — | 4 | 5 |
| 25–34 | 4 | 6 | 6 | 15 | 13 | 23 | 20 | 23 | 12 | 50 | 55 | 20 |
| 35–44 | 3 | 6 | 2 | 4 | 15 | 35 | 6 | 36 | 9 | 39 | 55 | 23 |
| 45–54 | 3 | 16 | 5 | 10 | 9 | 35 | 16 | 36 | 7 | 54 | 40 | 27 |
| 55–64 | — | — | 3 | 13 | 6 | 35 | 9 | 45 | 4 | 100 | 22 | 9 |
| 18–64 | 10 | 6 | 17 | 10 | 43 | 6 | 74 | 30 | 32 | 47 | 176 | 22 |
| 2 Phlegm in the morning, summer | | | | | | | | | | | | |
| 18–24 | — | — | 1 | 10 | — | — | 1 | 4 | — | — | 2 | 3 |
| 25–34 | 3 | 5 | 6 | 15 | 10 | 18 | 17 | 20 | 10 | 42 | 46 | 17 |
| 35–44 | 1 | 2 | 1 | 2 | 13 | 30 | 21 | 29 | 6 | 26 | 42 | 18 |
| 45–54 | 3 | 16 | 3 | 6 | 8 | 31 | 13 | 30 | 5 | 38 | 32 | 21 |
| 55–64 | — | — | 2 | 9 | 4 | 24 | 8 | 40 | 3 | 75 | 17 | 22 |
| 18–64 | 7 | 4 | 13 | 8 | 35 | 21 | 60 | 24 | 24 | 35 | 139 | 17 |
| 3 Phlegm all day winter | | | | | | | | | | | | |
| 18–24 | — | — | 1 | 10 | — | — | 1 | 4 | — | — | 2 | 3 |
| 25–34 | 3 | 5 | 4 | 10 | 7 | 12 | 13 | 15 | 7 | 29 | 34 | 12 |
| 35–44 | 3 | 6 | 2 | 4 | 10 | 3 | 18 | 25 | 7 | 30 | 40 | 17 |
| 45–54 | 3 | 16 | 3 | 6 | 6 | 23 | 9 | 20 | 3 | 23 | 24 | 16 |
| 55–64 | — | — | 1 | 4 | 4 | 24 | 1 | 5 | 1 | 25 | 7 | 9 |
| 18–64 | 9 | 6 | 11 | 6 | 27 | 16 | 42 | 17 | 18 | 26 | 107 | 13 |
| 4 Phlegm all day summer | | | | | | | | | | | | |
| 18–24 | — | — | 1 | 10 | — | — | 1 | 4 | — | — | 2 | 3 |
| 25–34 | 3 | 5 | 5 | 12 | 4 | 7 | 9 | 10 | 6 | 25 | 27 | 10 |
| 35–44 | 1 | 2 | 1 | 2 | 9 | 21 | 11 | 15 | 4 | 17 | 6 | 11 |
| 45–54 | 2 | 11 | 2 | 4 | 4 | 15 | 6 | 14 | 1 | 8 | 15 | 10 |
| 55–64 | — | — | 1 | 4 | 2 | 12 | 1 | 5 | 1 | 25 | 5 | 7 |
| 18–64 | 6 | 4 | 10 | 6 | 19 | 11 | 28 | 11 | 12 | 18 | 75 | 9 |
| 5 Phlegm 3 months in the year | | | | | | | | | | | | |
| 18–24 | — | — | 2 | 20 | — | — | 6 | 26 | — | — | 8 | 11 |
| 25–34 | 8 | 12 | 8 | 0 | 18 | 32 | 28 | 33 | 12 | 50 | 74 | 27 |
| 35–44 | 6 | 13 | 3 | 6 | 18 | 42 | 28 | 38 | 9 | 39 | 64 | 27 |
| 45–54 | 3 | 16 | 5 | 10 | 11 | 42 | 21 | 48 | 8 | 62 | 48 | 32 |
| 55–64 | — | — | 4 | 17 | 8 | 47 | 10 | 50 | 4 | 100 | 26 | 34 |
| 18–64 | 17 | 11 | 22 | 13 | 55 | 33 | 93 | 38 | 33 | 49 | 220 | 27 |

in the series under study The number of those suffering from asthma or airways obstruction is small six men were asthmatics whose disease had been diagnosed at hospital and had not been cured by the time the study was started Chronic bronchitis is more prevalent among smokers than non smokers and the frequency seems to increase with the amount smoked per day Age appears to play a part in the prevalence of CNSLD but if analysis is limited to the groups of non smokers and ex-smokers the effect of age does not appear

Pipe smokers were found to have chronic bronchitis more often than cigarette smokers but the difference was not significant.

Table IX and Figs 2–3 show the results of lung function tests as regression equations The $FEV_1$ and FVC are expressed as a function of age and height the latter had no significant effect on the $FEV_1$ which is therefore recorded only as a function of age

As far as forced vital capacity is concerned, there are no significant differences between the non-smokers and the various groups of smokers although the FVC diminishes most steeply with age in the group of those smoking at least 25 g/

Table VI *Prevalence of dyspnoea in various age and smoking groups*

No man had dyspnoea grade V

| | Non smokers | | Ex-smokers | | Smokers (g day) 1–14 | | 15–24 | | 25– | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No | | No | | No | | No | | No | | No | |
| 1 Dyspnoea grade II | | | | | | | | | | | | |
| 18–24 | — | — | 1 | 10 | — | — | 1 | 4 | 1 | 25 | 3 | 4 |
| 25 34 | 1 | 2 | — | — | 2 | 4 | 12 | 14 | 5 | 21 | 20 | 7 |
| 35–44 | 3 | 6 | 8 | 16 | 7 | 16 | 5 | 7 | 5 | 22 | 28 | 12 |
| 45–54 | — | — | 7 | 15 | 5 | 19 | 8 | 18 | — | — | 0 | 13 |
| 55–64 | 1 | 8 | 5 | 22 | 2 | 12 | 5 | 25 | — | — | 13 | 17 |
| 18–64 | 5 | 3 | 21 | 12 | 16 | 10 | 31 | 13 | 11 | 16 | 84 | 10 |
| 2. Dyspnoea grade III | | | | | | | | | | | | |
| 18 24 | — | — | — | — | — | — | — | — | — | — | — | — |
| 25–34 | — | — | 1 | 2 | 1 | 2 | — | — | — | — | 2 | 1 |
| 35–44 | — | — | 1 | 2 | 2 | 5 | 4 | 5 | 2 | 9 | 9 | 4 |
| 45–54 | 2 | 11 | — | — | 3 | 12 | 3 | 7 | 1 | 8 | 9 | 6 |
| 55–64 | 1 | 8 | 2 | 9 | 1 | 6 | 3 | 15 | — | — | 7 | 9 |
| 18–64 | 3 | 2 | 4 | 2 | 7 | 4 | 10 | 4 | 3 | 4 | 27 | 3 |
| 3 Dyspnoea grade IV | | | | | | | | | | | | |
| 18–24 | — | — | — | — | — | — | — | — | — | — | — | — |
| 25–34 | — | — | — | — | — | — | 1 | 1 | — | — | 1 | 0 |
| 35–44 | — | — | 1 | 4 | 1 | 2 | 2 | 3 | — | — | 4 | 2 |
| 45–54 | — | — | 3 | 6 | — | — | 2 | 5 | 1 | 8 | 6 | 4 |
| 55–64 | — | — | 1 | 4 | 2 | 12 | 3 | 15 | — | — | 6 | 1 |
| 18–64 | — | — | 5 | 3 | 3 | 2 | 8 | 3 | 1 | 1 | 17 | |

day In regard to the $FEV_1$ the differences are distinct when the regression equation based the total material is compared with the corresponding equations obtained for the various smoking groups the differences are found to be almost significant ($P < 0.05$) The differences arise mainly from the disparity of the age coefficients though these do not differ significantly one from another The injurious effect of smoking on ventilatory function appears most strikingly in the equations for the $FEV_1$ in which the differences are highly significant ($P < 0.001$) The ventilatory capacity of ex smokers in this series is intermediate between the non smokers and smokers

*Other findings*

A comparison between the relatively small group of chlorine factory workers (about 90 men) and the rest of the population reveals differences neither in the prevalence of respiratory symptoms or of CNSLD nor in pulmonary function tests. Five per cent of the healthy subjects and 8% of those with diagnosed CNSLD were suffering or had suffered from peptic ulcer Such ulcers were more frequently found in smokers than in non-smokers, yet the differences are not significant.

## DISCUSSION

The *methods and diagnostic criteria* have bee described in detail in an earlier paper (10) Th group of asthma/airways obstruction does no form any well characterized diagnostic category Obviously many of these subjects have pulmonar emphysema which nowadays is most properl diagnosed in anatomical terms (1 18 31) Wit the available methods and facilities it was no however possible to establish without doubt th diagnosis of emphysema, nor could the subjec be requested to attend a closer clinical examina tion Imell and Kiviloog (12) also thought it u realistic to make a diagnosis of emphysema in survey of this kind so they diagnosed these pa tients as having asthma this too may give ri to some confusion if the diagnostic criteria ar not clearly set forth.

The smoking habits appear to be much the sam as in the surveys published earlier from Finlan

Table VII *Prevalence of other respiratory symptoms in various age and smoking groups*

| | Non smokers | | Ex smokers | | Smokers (g/day) 1–14 | | 15–24 | | 25– | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No | | No | | No | | No | | No | | No | |
| 1 Wheezing | | | | | | | | | | | | |
| 18–24 | 1 | 6 | 1 | 10 | 3 | 13 | 5 | 22 | — | — | 10 | 13 |
| 25–34 | 6 | 9 | 6 | 15 | 16 | 8 | 0 | 23 | 9 | 38 | 57 | 21 |
| 35–44 | 2 | 4 | 5 | 10 | 11 | 6 | 16 | 22 | 11 | 48 | 45 | 19 |
| 45–54 | 5 | 6 | 9 | 19 | 2 | 8 | 13 | 30 | 5 | 38 | 34 | 23 |
| 55–64 | 1 | 8 | 6 | 26 | 7 | 41 | 5 | 25 | 1 | 25 | 0 | 6 |
| 18–64 | 15 | 9 | 27 | 16 | 39 | 23 | 59 | 24 | 26 | 38 | 166 | 20 |
| 2 Wheezing most days or nights | | | | | | | | | | | | |
| 18–24 | — | — | — | — | — | — | 1 | 4 | — | — | 1 | 1 |
| 25–34 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 1 | — | — | 4 | 1 |
| 35–44 | — | — | — | — | 2 | 5 | 2 | 3 | 2 | 9 | 6 | 3 |
| 45–54 | — | — | 2 | 4 | — | — | 3 | 7 | 1 | 8 | 6 | 4 |
| 55–64 | — | — | 1 | 4 | 1 | 6 | 3 | 15 | — | — | 5 | 7 |
| 18–64 | 1 | 1 | 4 | 2 | 4 | 2 | 10 | 4 | 3 | 4 | 22 | 3 |
| 3 Weather affects chest | | | | | | | | | | | | |
| 18–24 | 1 | 6 | 1 | 10 | — | — | 1 | 4 | — | — | 3 | 4 |
| 25–34 | 1 | 2 | 4 | 10 | 3 | 5 | 1 | 1 | 2 | 8 | 11 | 4 |
| 35–44 | 1 | 2 | 3 | 6 | 4 | 9 | 8 | 11 | 3 | 13 | 19 | 8 |
| 45–54 | 5 | 26 | 7 | 15 | 4 | 15 | 7 | 16 | 1 | 8 | 24 | 16 |
| 55–64 | — | — | 5 | 22 | 3 | 18 | 4 | 20 | — | — | 12 | 16 |
| 18–64 | 8 | 5 | 20 | 12 | 14 | 8 | 21 | 9 | 6 | 9 | 69 | 8 |
| 4 Nasal catarrh 3 months in the year | | | | | | | | | | | | |
| 18–24 | 4 | 25 | 1 | 10 | 3 | 13 | 3 | 13 | — | — | 11 | 14 |
| 25–34 | 8 | 12 | 8 | 20 | 6 | 11 | 15 | 17 | 6 | 25 | 43 | 16 |
| 35–44 | 6 | 13 | 4 | 8 | 5 | 12 | 9 | 12 | 5 | 22 | 9 | 12 |
| 45–54 | 3 | 16 | 7 | 15 | 5 | 19 | 9 | 20 | 1 | 8 | 25 | 17 |
| 55–64 | — | — | 1 | 4 | 2 | 12 | 2 | 10 | 1 | 25 | 6 | 8 |
| 18–64 | 21 | 13 | 21 | 12 | 21 | 13 | 38 | 15 | 13 | 19 | 114 | 14 |
| 5 Chest illness of at least one week's duration during the past 3 years | | | | | | | | | | | | |
| 18–24 | — | — | — | — | — | — | — | — | — | — | — | — |
| 25–34 | 1 | 2 | 2 | 5 | 3 | 5 | 5 | 6 | 1 | 4 | 12 | 4 |
| 35–44 | 2 | 4 | — | — | 2 | 5 | 7 | 10 | 1 | 4 | 12 | 5 |
| 45–54 | — | — | — | — | 4 | 15 | 4 | 9 | 1 | 8 | 9 | 6 |
| 55–64 | — | — | — | — | 2 | 12 | 2 | 10 | — | — | 4 | 5 |
| 18–64 | 3 | 2 | 2 | 1 | 11 | 7 | 18 | 7 | 3 | 4 | 37 | 5 |

(10, 22, 28). There were, however, more ex-smokers in the present study than in earlier populations, especially in the older age groups, and the number of men smoking 25 g daily or more was rather small. It is not clear whether this is due to the cold unfavourable climate, the industrial environment or other factors. It is understandable that factors like these may add to the injurious effects of smoking and thus make a man stop smoking or diminish the amount smoked at an earlier age than might be the case in more favourable circumstances. Non-smokers and especially ex-smokers are on average heavier than smokers—a finding that has been reported earlier (10).

Both cough and phlegm production increase with an increase in the amount smoked; ex-smokers are intermediate between non-smokers and smokers. These symptoms are definitely less frequent in summer than in winter. The prevalence of chronic bronchitis accords closely with that of phlegm production, which is clear from the definitions. Most of the CNSLD is due to chronic bronchitis, the prevalence of asthma/airways obstruction is low.

It is of some interest to compare the results of

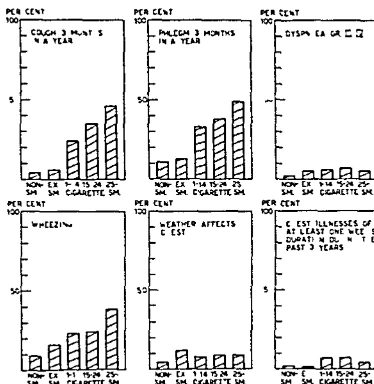



*Fig 1* Prevalence of main respiratory symptoms in the various smoking groups

Table VIII *Prevalence of chronic non specific lung disease (CNSLD) and its components in various age and smoking groups*

| | Non smokers | | Ex smokers | | Smokers (g day) 1–14 | | 15–24 | | 25– | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % | No | % | No | |
| **CNSLD** | | | | | | | | | | | | |
| 18 -4 | — | — | 2 | 0 | — | — | 6 | .6 | — | — | 8 | 11 |
| 25-34 | 8 | 1. | 8 | .0 | 18 | 32 | .8 | 33 | 12 | 50 | 74 | 17 |
| 35-44 | 7 | 15 | 3 | 6 | 19 | 44 | .8 | 38 | 9 | 39 | 66 | 8 |
| 45 54 | 3 | 16 | 8 | 17 | 1 | 46 | 21 | 48 | 8 | 62 | 52 | 35 |
| 55-64 | 1 | 8 | 4 | 17 | 11 | 65 | 13 | 65 | 4 | 100 | 33 | 43 |
| 18-64 | 19 | 1. | .5 | 15 | 60 | 36 | 96 | 39 | 33 | 49 | .33 | 9 |
| **Chronic bronchitis** | | | | | | | | | | | | |
| 18-.4 | — | — | 2 | .0 | — | — | 6 | .6 | — | — | 8 | 11 |
| 25 34 | 8 | 12 | 7 | 17 | 18 | 32 | .8 | 33 | 12 | 50 | 73 | 7 |
| 35-44 | 6 | 13 | 3 | 6 | 18 | 42 | .8 | 18 | 9 | 39 | 64 | 7 |
| 45 54 | 3 | 16 | 4 | 8 | 11 | 42 | .0 | 45 | 8 | 62 | 46 | 31 |
| 55-64 | — | — | 3 | 13 | 8 | 47 | 10 | 50 | 4 | 100 | .5 | 33 |
| 18-64 | 17 | 11 | 19 | 11 | 55 | 33 | 92 | 37 | 33 | 49 | 216 | 7 |
| **Asthma airways obstruction** | | | | | | | | | | | | |
| 18- 4 | — | — | — | — | — | — | — | — | — | — | — | — |
| .5-34 | — | — | 1 | 2 | — | — | — | — | — | — | 1 | 0 |
| 35-44 | 1 | [illegible] | — | — | 1 | 2 | — | — | — | — | [illegible] | 1 |
| 45-54 | — | — | 4 | 8 | 1 | 4 | 3 | 7 | — | — | 8 | 5 |
| 55-64 | 1 | 8 | 1 | 4 | 3 | 18 | 3 | 15 | — | — | 8 | 11 |
| 18-64 | 2 | 1 | 6 | 3 | 5 | 3 | 6 | 2 | — | — | 19 | [illegible] |

Table IX *Regression equations for the various indices of ventilatory function*

Height in m age in y
R S D = residual standard deviation

| | Regression coefficients | | | |
|---|---|---|---|---|
| | Height | Age | Constant | R.S.D |
| Non smokers | | | | |
| $FEV_1$ | 6 05 | −0 036 | −5 03 | 0 50 |
| FVC | 7 95 | −0 030 | −7 41 | 0 59 |
| $FEV_1$ | — | −0 4 | 87 6 | 5 9 |
| Ex smokers | | | | |
| $FEV_1$ | 5 19 | −0 044 | −3 36 | 0 54 |
| FVC | 6 73 | −0 038 | −5 07 | 0 61 |
| $FEV_1$ | — | −0 31 | 88 4 | 6 8 |
| Smokers, 1 14 g day | | | | |
| $FEV_1$ | 5 34 | −0 039 | −3 79 | 0 45 |
| FVC | 7 53 | −0 025 | −6 84 | 0 53 |
| $FEV_1$ | — | −0 41 | 90 9 | 6 5 |
| Smokers 15–24 g/day | | | | |
| $FEV_1$ | 5 03 | −0 044 | −3 12 | 0 51 |
| FVC | 7 12 | −0 031 | −6 00 | 0 58 |
| $FEV_1$ | — | −0 42 | 91 7 | 6 9 |
| Smokers 25+ g/day | | | | |
| FEV | 4 66 | −0 052 | −2 33 | 0 50 |
| FVC | 6 9? | −0 040 | −5 39 | 0 58 |
| $FEV_1$ | — | −0 39 | 89 1 | 6 8 |

the present study with those obtained by Huhti (10) in a rural population in Western Finland (Table X) In almost all symptom groups with the exception of temporary chest illnesses the prevalence figures are higher in Oulu When non smokers only are considered the difference is even more striking The smoking habits differ slightly in the two populations and the number of non smokers is small hence significance tests are meaningless The same interviewer was not used in the two studies and though the technique of investigation was as far as possible standardized observer variation must be taken into account If the differences observed are real ones they may be attributable to the climate or to the damaging effect of the industrial environment The fact that among the chlorine workers respiratory symptoms were not more frequent than in the rest of the series suggests that weather conditions might be a factor in the different prevalence rates It remains undetermined why temporary chest ill nesses were more prevalent in Harjavalta If the climate and/or industrial factors played a part the difference would be assumed to be in the same direction as in the case of respiratory symptoms proper At Harjavalta the history given by the patients themselves was relied upon when record ing the results This may make them less depend able than in Oulu where all absences from work were checked on the basis of each subject s health record card Social factors too may con tribute to the difference

Poppius et al (22) have investigated the preva lence of respiratory symptoms in Finnish lumber jacks their series consisted of 1231 m n 975 being

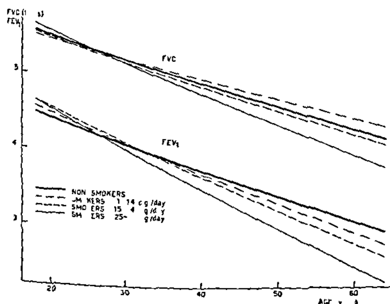



*Fig 2* FEV and FVC in non smokers and various groups of smokers Height standardized to 170 cm.

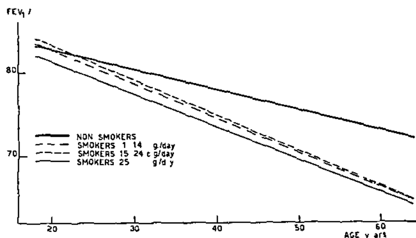



*Fig 3* FEV l in non smokers and various groups of smokers

included in the analysis proper A comparison with the present survey disclosed no essential differences in respect of cough and phlegm production However the amount smoked by the lumberjacks was greater and they included less non smokers Taking the smoking habits into consideration phlegm production was slightly more common among the industrial workers Dyspnoea *was more prevalent among the lumberjacks* Wheezing was equally frequent in both studies Among lumberjacks temporary chest illnesses increased in frequency with age in this respect age not found to play a part in the Oulu survey

The prevalence of respiratory symptoms and sease has claimed much attention in other countries but there are only few studies which lend themselves to a meaningful comparison with the present survey The age classification may be different from the one used in Oulu smoking habits are not stated according to the same pattern and the population may be differently chosen The relationship between CNSLD and various occupations has been discussed at some length elsewhere (10) and later attention has been drawn especially by Lowe (15) to the fact that jobs involv*ing exposure to heavy atmospheric pollution carry* an increased risk of bronchitis The single occupational group in which this has obviously been established beyond doubt is that of coalminers for other occupations the question is still open In the present investigation the deleterious effect of the industrial environment is not clearly recognizable In a number of studies the prevalence rates for cough and phlegm production have proved relatively similar but wheezing and especially the frequency of respiratory illnesses during the preceding three years have been higher than in Oulu (5 9 19 24) All these studies included fewer non smokers and more smokers than the present one However the report from Berlin (USA) by Ferris and Anderson (3) resembled the Oulu survey very much in regard to both smoking habits and prevalence of symptoms It is evident that smoking dominates as a cause of respiratory symptoms whereas the climate and occupation play a less important part

Three extensive studies on the prevalence of CNSLD have recently been published in Sweden (12 13 29) The prevalence figures in all these are considerably lower than those found in Finland and most other countries Wilhelmsen and Tibblin (29) reported the prevalence of chronic

Table X *The percentages of respiratory symptoms and chronic bronchitis in two different populations in Finland*

Males only Age group 45–64 y

| | Western Finland (Huhti 1965) | | Northern Finland (Present study) | |
|---|---|---|---|---|
| | Non smokers | Total | Non smokers | Total |
| Cough 3 months a year | 3 | 26 | 7 | 30 |
| Wheezing | 9 | 19 | 21 | 24 |
| Effect of weather | 4 | 14 | 17 | 16 |
| Dyspnoea grade III | 4 | 7 | 10 | 7 |
| Temporary chest illnesses | 12 | 14 | 0 | 6 |
| Chronic bronchitis | 7 | 29 | 10 | 31 |

bronchitis among 50 year-old men in Gothenburg to be 7 6% Julin and Wilhelmsen (13) also studied men (and women) in Gothenburg report ing the prevalence of chronic bronchitis as 2 1% in men aged 16–64 years According to Irnell and Kiviloog (12) the prevalence of CNSLD in Uppsala and its surroundings was 5% for men aged 30–64 years Variations in smoking habits do not explain the observed differences though they con tribute to the discrepancy In part, the differences may be due to causes relating to examination technique in part to the high standard of living in Sweden There is some evidence that bronchitis morbidity may be higher in the lower social groups (2 20) Such results however have only been reported from Great Britain and have not been confirmed elsewhere neither did Irnell and Kiviloog in their report find CNSLD prevalence to be affected by social status Since Sweden and Finland are closely situated geographically it would be interesting to carry out a comparative well standardized population survey in both coun tries with the aim of determining the extent to which real differences exist

The present investigation confirms the previous finding that smokers have poorer ventilatory func tion than non smokers Ex smokers seem to be intermediate between these groups In them it is a matter of obstructive changes in the airways which are most clearly apparent in the fact that the smokers show a steeper decrease of $FEV_1$ as age increases In forced vital capacity th re are no significant differences between the smoking categories The regression equations for the $FEV_1$ based on the Oulu series seem to agree very well with the results of the Harjavalta survey (11) but the FVC is slightly lower in the latter As recorded by Poppius (21) the normal values for the $FEV_1$ are lower and FVC diminishes more steeply with age than in the present study This latter factor is probably the reason why his nor mal values of $FEV_{1.0}$ in the older age groups are distinctly higher than in the series under study

Those affected with CNSLD were not found in the present study to have peptic ulcer signifi cantly more often than the healthy subjects the result thus being the same as in the series from Harjavalta It is possible of course that the number of cases was not large enough for the difference to become significant results opposed to these have been reported (8 23 30) Autopsy findings have been along the same lines as the results of the present work


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## REFERENCES


1 Ciba Guest Symposium Terminology definitions, and classification of chronic pulmonary emphysema and related conditions. Thorax 14 286 1959
2 College of General Practitioners Chronic bronchitis in Great Britain. A national survey carried out by the Respiratory Diseases Study Group of the College of General Practitioners. Brit. med J — 973 1961
3 Ferris, B G & Anderson D O The prevalence of chronic respiratory disease in a New Hampshire town Amer Rev resp Dis. 86 165 1962
4 Fletcher C M The clinical diagnosis of pulmonary emphysema. An experimental study Proc roy Soc Med 45 577 1952.
5 Fletcher C M Elmes P C Fairbairn, A S & Wood, C H The significance of respiratory symptoms and the diagnosis of chronic bronchitis in a working population Brit med J 2 257 1959
6 Gilson, J C & Hugh Jones, P Lung function in coalworkers pneumoconiosis. Spec Rep Ser med. Res Coun (Lond) No 290 Her Majesty s Station ery Office London 1955
7 Higgins, I T T Cochrane A. L. Gilson J C & Wood, C H Population studies of chronic respira tory disease a comparison of miners, foundry work ers, and others in Staveley Derbyshire Brit J industr Med. 16 255 1959
8 Hirvonen, M Klinisch-statistische Untersuchungen uber das chronische Lungenemphysem. Acta med scand. 108 73 1941
9 Holland, W W & Stone R. W Respiratory disorders in United States East Coast telephone men Amer J Epidem. 82 92 1965
10 Huhti, E. Prevalence of respiratory symptoms, chronic bronchitis and pulmonary emphysema in a Finnish rural population. Field survey of age group 40–64 in the Harjavalta area. Acta tuberc pneumol scand Suppl 61 1965
11 — Ventilatory function in healthy non-smokers and smokers. Scand. J resp Dis 48 149 1967
12 Irnell L & Kiviloog, J Bronchial asthma and chronic bronchitis in a Swedish urban and rural population. Scand J resp Dis Suppl 66 1968
13 Julin A & Wilhelmsen L. Bronchial asthma and chronic bronchitis in a random population sample Scand J resp Dis 48 330 1967
14 Linder A. Statistische Methoden fur Naturwissenschaftler Mediziner und Ingenieure Fourth ed., p 185 Birkhauser Verlag, Basel 1964

15 Lowe C R. Chronic bronchitis and occupation Proc roy Soc. Med 61 98 1968
16 McKerrow C B., McDermott, M & Gilson J C A spirometer for measuring the forced expiratory volume with a simple calibrating device Lancet 1 149 1960
17 Medical Research Council s Committee on the Aetiology of Chronic Bronchitis Standardized questionnaires on respiratory symptoms Brit med J 2 1665 1960
18 Meneely G R Renzetti, A. D Steele J D Wyatt, J P & Harris H W Definitions and classification of chronic bronchitis, asthma and pulmonary emphysema. Amer Rev resp Dis 85 762 1962
19 Mork, T A comparative study of respiratory disease in England and Wales and Norway Acta med scand Suppl. 384 1962
20 Ogilvie A. G & Newell, D J Chronic bronchitis in Newcastle upon Tyne Livingstone Edinburgh 1957
21 Poppius H Airway obstruction distribution of inspired gas and physiologic dead space in pulmonary tuberculosis Ann Acad. Sci fenn. A5 No 114 1965
22. Poppius, H., Lehtinen M & Patiala, J Chronic bronchitis in forest workers in Finland Ann Med intern Fenn. 54 1 1965
23 Reid D D & Fairbairn, A. S The natural history of chronic bronchitis Lancet 1 1147 1958
24 Sharp J T Paul O Lepper M H. McKean, H. & Saxton, G A. Prevalence of chronic bronchitis in an American male urban industrial population Amer Rev resp Dis 91 510 1965
25 Stuart Harris, C H Chronic bronchitis I Abstr Wld Med 42 649 1968
26 — Chronic bronchitis II Abstr Wld Med 42 737 1968
Thurlbeck, W M A clinico pathological study of emphysema in an American hospital. Thorax 18 59 1963
28 Tuomi, E Prevalence of various heart diseases and high blood pressure in Kaikala (a rural commune in Finland) Duodecim (Helsinki) Suppl 45 1965
29 Wilhelmsen, L & Tibblin, G Tobacco smoking in fifty year-old men I Respiratory symptoms and ventilatory function tests Scand J resp Dis 47 121 1966
30 Williams, M J & Mendel J L Pulmonary emphysema, adrenocortical hyperplasia and peptic ulceration Dis Chest 44 303 1963
31 World Health Organization Chronic cor pulmonale Report of an Expert Committee Wld Hlth Org. techn Rep Ser 213 1961

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# MUSCLE GLYCOGEN IN PATIENTS WITH DIABETES MELLITUS

*Glycogen Content before Treatment and the Effect of Insulin*

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*Abstract* The glycogen concentration of *m quadriceps femoris* has been determined in 54 cases of newly detected untreated diabetes between the ages of 2 and 73 years. Muscle samples were taken by needle biopsy technique The muscle glycogen values of the diabetics were compared to the values of 26 hospitalized non-diabetic patients within the same ages and to a normal material of 228 healthy volunteers According to the clinical findings the patient material was divided into three groups juvenile diabetics, insulin-dependent adults and adults independent of insulin The following results were found

1 Compared to the values of the control material significantly decreased muscle glycogen was found in all of the types of diabetes examined.

2. The mean glycogen values of the three diabetic groups were significantly different.

3 Apparently there is a relation between the severity of diabetic disease and the muscle glycogen concentration in untreated cases, the lowest values being found in juvenile diabetes and the less decreased values in maturity-onset diabetes.

4 During insulin treatment a rapid increase of the muscle glycogen was observed, especially during the first three days.

5 The results indicate that a peripheral lack of insulin efficacy is the most important cause of low muscle glycogen concentration in untreated diabetes, the details, however still being obscure

As early as 1890 von Mering and Minkowsky (21) pointed out that the glycogen content of both muscle and liver during experimental diabetes was low Fisher and Lackey (10) showed that the muscle and liver glycogen of starving pancreatectomized dogs decreased faster than that of normal starving animals The feeding of carbohydrates following starvation resulted in a considerably lower increase in the glycogen of the diabetic animals than in the normal ones After insulin administration it was found that the levels of glycogen in the diabetic dogs were approximately the same as those in the normal dogs

Since 1940 when Gemmil (11) first succeeded in demonstrating an increase of glucose uptake and glycogen synthesis in isolated muscle following the addition of insulin there have been many other reports of in vitro studies on glycogen synthesis in muscle (12 18 19 20 22 24 25) However direct determinations of glycogen in muscle tissue from human diabetic subjects have only rarely been reported (4 5 14 23) Hildes et al (14) could not find any significant difference between the glycogen values of treated diabetics who had been deprived of insulin for two days and those of normal individuals Both in the previous study and that of Beringer (5 14) on muscle glycogen in diabetic patients undergoing treatment no mention was made of the values for glycogen prior to treatment In a study including six cases of untreated juvenile diabetics we found a low content of glycogen in muscle (4) During subsequent treatment of these cases there was a rapid increase of the glycogen content Decreased glycogen content in diabetes has also recently been reported by Naccarato et al (23)

In the present investigation 54 cases of previously untreated diabetes were examined The patient material includes children as well as adults and their conditions ranged from cases totally dependent on insulin to cases independent of insulin Our intention has been to study whether the

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Table I *Juvenile diabetes Analytical values before and during insulin treatment*

| Case no | Sex | Age | Ht (cm) | BW (kg) | Duration of symptoms | Fasting blood glucose (mg/100 ml) | Urine glucose (g/24 h) | Ketone bodies in urine | Bicar bonate (mmol/l) | Muscle glycogen (g/100 g wet weight) | Insulin treatment Duration | I U / 24 h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ♂ | 13 | 167 | 42 0 | 1 mo | 232 | 144 | + + | 13 0 | 0 52 | 0 | 0 |
| | | | | 44 0 | | 118 | 5 | 0 | — | 1 32 | 14 days | 20 |
| 2 | ♂ | 6 | 118 | 18 7 | 1½ week | 170 | 72 | + + + | 14 0 | 0 73 | 0 | 0 |
| | | | | 19 5 | | 115 | 0 | (+) | — | 1 76 | 9 days | 8 |
| 3 | ♂ | 15 | 170 | 48 5 | 3 weeks | 234 | 94 | + + + | 13 0 | 0 54 | 0 | 0 |
| | | | | 50 9 | | 116 | 51 | 0 | — | 0 93 | 24 h | 00 |
| | | | | 54 8 | | 183 | 31 | 0 | 24 8 | 1 94 | 13 days | 32 |
| 4 | ♀ | 12 | 146 | 27 8 | 1½ week | ѧ00 | — | + + + | 22 0 | 0 35 | 0 | 0 |
| | | | | — | | 167 | 17 | (+) | — | 0 86 | 42 h | 60 |
| | | | | 30 0 | | 117 | 2 | 0 | — | 0 84 | 21 days | 16 |
| 5 | ♂ | 5 | 105 | 16 0 | 1½ week | 276 | 53 | + + | 20 0 | 0 40 | 0 | 0 |
| | | | | — | | 145 | 24 | 0 | — | 1 92 | 3 days | ⁷8 |
| | | | | 17 5 | | 162 | 31 | 0 | — | 2 00 | 13 days | 6 |
| 6 | ♀ | 12 | 142 | 33 7 | 3 mo | 245 | 74 | + + + | — | 0 50 | 0 | 0 |
| | | | | — | | 278 | 53 | 0 | — | 2 17 | 24 h | 100 |
| | | | | 34 9 | | 145 | 21 | 0 | — | 1 07 | 15 days | 0 |
| 7 | ♂ | 3 | 87 | 10 7 | 2 weeks | 187 | 30 | + + + | 19 0 | 0 48 | 0 | 0 |
| | | | | 11 5 | | 69 | 7 | 0 | — | 1 74 | 3 days | 16 |
| 8 | ♀ | 5 | 110 | 17 4 | 1 mo | 210 | 192 | + + | 19 6 | 0 29 | 0 | 0 |
| | | | | 18 2 | | 86 | 9 | 0 | – | 0 78 | 4 days | 28 |
| 9 | ♂ | 11 | 147 | 36 0 | 2 mo | 268 | 139 | + + + | 16 0 | 0 62 | 0 | 0 |
| | | | | — | | ѧ85 | 41 | + + | — | 1 24 | 24 h | 60 |
| | | | | 38 6 | | 100 | 0 | 0 | 22 0 | 1 74 | 15 days | 16 |
| 10 | ♂ | 11 | 144 | 32 5 | 1 y | 237 | 156 | + + + | — | 0 45 | 0 | 0 |
| | | | | 35 8 | | 175 | 0 | 0 | — | 2 42 | 19 days | 24 |
| 11 | ♂ | 2 | 80 | 11 3 | 3 weeks | 115[a] | 18 | + + | — | 0 18 | 0 | 0 |
| 12 | ♂ | 6 | 116 | 19 1 | 3 weeks | 110[b] | 14 | + + | 13 0 | 0 84 | 0 | 0 |

$n = 12$
glycogen Mean 0 49 g/100 g wet weight range 0 18–0 84
At biopsy (10 a m ) 228 mg/100 ml (only one biopsy performed)
At biopsy (10 a m ) 285 mg/100 ml (only one biopsy performed)

severity of the diabetic condition is reflected in the muscle glycogen content of the patient prior to treatment The subsequent effect of insulin treatment on the muscle glycogen content was also followed

## MATERIAL AND METHODS

1 *Patients*

The investigation comprised only patients with untreated manifest diabetes mellitus Cases of prediabetes or latent diabetes were not included Patients with a history or with symptoms of other metabolic disturbances gastro intestinal diseases malnutrition or serious circulatory disturbances were excluded All patients were hospitalized during the study

*Juvenile diabetes* Nine boys and three girls from 2 to 15 years old and with newly discovered untreated dia betes were examined According to their histories symp toms had occurred during periods varying from ten days to three months with the exception of one case in which light symptoms had occurred sporadically over one year All patients had ketonuria and low values of serum bicarbonate These patients were considered insulin-depen dent from the beginning and this was confirmed by the ubsequent development of the disease Anthropometric and clinical data will be found in Table I

*Adult diabetes* Forty two untreated patients ranging from 20 to 73 years old were examined The degree of severity of the disease affecting patients in this group varied greatly The patients were divided into an insulin dependent group and a non insulin dependent group

*Insulin dependent patients* (Table II) This group com prised twelve subjects six men and six women aged from ѧ0 to 71 years According to their histories symptoms of diabetes had occurred in only two cases for more than six months Ketonuria was found in none of the twelve cases As a result of the rapid progression of the disease in these patients which was characterized by a s vere loss of weight considerable glycosuria and other clinical find ings these patients were judged to be primarily insulin dependent The insulin treatment was not later replaced by oral treatment

*Non insulin depend nt patients* (Table III) This group comprised thirty patients thirteen women and seventeen men aged from 24 to 73 years Since only three of the

Table II *Adult insulin-dependent diabetes Analytical values before and during insulin treatment*

| Case no | Sex | Age | Ht (cm) | BW (kg) | Duration of symptoms | Fasting blood glucose (mg/100 ml) | Urine glucose (g/24 h) | Ketone bodies in urine | Bicarbonate (mmol l) | Muscle glycogen (g 100 g wet weight) | Insulin treatment Duration | I U / 24 h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13 | o | 71 | 158 | 44 2 | 6 mo | ₋60 | 62 | 0 | — | 0 98 | 0 | 0 |
| | | | | 44 0 | | 130 | 40 | + + | — | 1 06 | 4 h | 52 |
| | | | | 44 6 | | 170 | 6 | 0 | — | 1 46 | 8 days | 8 |
| 14 | o | 41 | 187 | 80 7 | 6 mo | 190 | 21 | + | ₋6 0 | 0 66 | 0 | 0 |
| | | | | — | | 175 | 18 | (+) | — | 1 00 | 24 h | 40 |
| 15 | ♀ | 54 | 163 | 0.2 | 9 mo | 310 | 70 | 0 | 29 0 | 0 75 | 0 | 0 |
| | | | | 71 0 | | 2₋0 | — | 0 | — | 1 ₋8 | 48 h | 3₋ |
| | | | | 73 0 | | 100 | 0 | 0 | — | 1 71 | 10 days | 32 |
| 16 | ♀ | 59 | 168 | 64 6 | 4 mo | 250 | 49 | 0 | — | 0 77 | 0 | 0 |
| | | | | — | | 190 | 27 | (+) | — | 1 05 | 24 h | 92 |
| | | | | 66 5 | | 130 | 0 | 0 | — | 1 49 | 12 days | 24 |
| 17 | o | 50 | 169 | 75 0 | 1 y | 273 | 94 | 0 | — | 1 01 | 0 | 0 |
| | | | | — | | 163 | 44 | 0 | — | 1 62 | 3 days | 40 |
| | | | | 76 3 | | 190 | 27 | 0 | — | 1 45 | 9 days | 36 |
| 18 | o | 63 | 175 | 80 7 | 1 week | 335 | 61 | — | 31 0 | 0 88 | 0 | 0 |
| | | | | 81 0 | | 290 | 57 | — | — | 1 37 | ?4 h | 80 |
| | | | | 82.5 | | 180 | 11 | 0 | — | 1 47 | 4 days | 60 |
| 19 | ♂ | 46 | 180 | 75 1 | 1 mo | ₋00 | 1₋0 | + + + | 22 0 | 0 69 | 0 | 0 |
| | | | | 77 1 | | 140 | 17 | 0 | 28 0 | 1 63 | 7 days | 64 |
| | | | | 77 0 | | 140 | 3 | 0 | 27 0 | 1 35 | 14 days | 40 |
| 0 | ♀ | 0 | 167 | 46 0 | 1½ week | 5 0 | 195 | + + + | 21 0 | 0 36 | 0 | 0 |
| | | | | 51 0 | | ₋80 | 33 | 0 | 31 0 | 1 48 | 4 days | 80 |
| | | | | 53 0 | | 220 | (+) | 0 | — | 1 57 | 1? days | ₋4 |
| 1 | ♀ | 58 | 154 | 72.4 | 1 mo | 290 | 80 | + + + | 29 0 | 0 77 | ([a]) | |
| ₋2 | ♀ | 23 | 159 | 51 7 | 2 mo | 130 | 41 | + + | 21 0 | 1 06 | ([b]) | |
| 23 | ♂ | ? | 181 | 73 6 | 2 mo | 245 | 100 | + + + | 30 0 | 0 80 | ([b]) | |
| 4 | o | 21 | 168 | 48 5 | 2 weeks | 235 | 116 | + + + | 8 0 | 0 59 | ([b]) | |

$n = 12$.
Basal glycogen Mean 0 78 g/100 g wet weight range 0 36–1 06
[a] Only one biopsy performed
[b] Spec al treatment.

patients were under 40 years of age the majority of the cases were thus defined as maturity-onset diabetes. Ke tonuria was found in only four cases and all responded fa ourably to oral treatment combined with diet restric tions. Twenty-one patients were treated with sulphonylurea compounds (tolbutamide carbutamide, chlorpropamide and glu̇odiazine) nine patients were treated with a diguanide (Metformin) Only the basic glycogen values, i.e values before treatment, of the orally treated patients are pre sented. The blood bicarbonate values before treatment were normal.

*₋ Diet*

The juvenile diabetics were subjected to a calorie and carbohydrate regime which was adjusted with respect to the r size and age The adult patients were given a diet containing 1700 to 2100 calories per day 460 to 680 (mean 560) of which consisted of carbohydrates.

3 *Contr l material*

Ear er reports of normal glycogen values have varied considerably depending partly on which muscle groups were examined and the biopsy technique and method of an lysis used. Normal muscle glycogen values in 2₋8 healthy indi iduals, examined according to the methods used in this study were recently published by Hultman (16) The age distr bution of this group varied from 18 to 40 years.

In order to obtain a control material comparable to the diabetics two groups of hospitalized patients were examined. One group in luded ten children from 3 to 15 years, and the other sixteen adults from 39 to 78 years of age These patients had no known diabetic heredity and l cked any history of metabolic or gastro-intestinal disturbances. On repeated examination only normal fasting blood sugar levels were found, and no glucose was detected in the urine The patients had been in hospital for 2 to 7 days before muscle biops es were performed

4 *Methods*

In most of the cases one initial biopsy was made for glycogen determination one to three days after admission to hosp tal In a few cases, where treatn ent was considered ur₋ent, a biopsy was made on the first day of hospitalization and, as in all other cases, before any treatment was started With the exception of the urgent cases mentioned above all biopsies were made in the

Table III *Adult non insulin dependent diabetes Analytical values before oral treatment*

| Case no | Sex | Age | Ht (cm) | BW (kg) | Duration of symptoms | Fasting blood glucose (mg/100 ml) | Urine glucose (g/24 h) | Ketone bodies in urine | Muscle glycogen (g/100 g wet weight) |
|---|---|---|---|---|---|---|---|---|---|
| 25 | ♀ | 53 | 161 | 91 4 | 1 y | 160 | 101 | 0 | 1 02 |
| 26 | ♀ | 73 | 159 | 54 5 | 3½ y | 210 | 32 | 0 | 1 14 |
| 27 | ♂ | 60 | 178 | 67 4 | 6 mo | 160 | 57 | 0 | 1 15 |
| 28 | ♂ | 65 | 165 | 55 3 | 4 y | 240 | 44 | 0 | 1 21 |
| 29 | ♂ | 50 | 190 | 78 0 | 10 y | 160 | 88 | 0 | 1 07 |
| 30 | ♀ | 71 | 161 | 62 3 | 2 mo | 255 | 21 | 0 | 0 96 |
| 31 | ♀ | 52 | 158 | 78 0 | 2 mo | .00 | 8 | + + | 0 99 |
| 32 | ♂ | 46 | 177 | 70 0 | 6 mo | 160 | 37 | + | 1 11 |
| 33 | ♀ | 61 | 161 | 53 2 | 1 y | 230 | 47 | + + + | 0 96 |
| 34 | ♀ | 40 | 156 | 78 6 | 6 mo | 280 | 14 | 0 | 0 77 |
| 35 | ♀ | 59 | 165 | 88 0 | 3 mo | 195 | 18 | 0 | 1 29 |
| 36 | ♀ | 70 | 168 | 72.5 | 3 mo | 312 | 25 | 0 | 1 12 |
| 37 | ♀ | 52 | 165 | 88 5 | 5 mo | 170 | 24 | 0 | 1 27 |
| 38 | ♂ | 61 | 180 | 101 0 | 1½ y | 165 | 5 | 0 | 1 34 |
| 39 | ♂ | 67 | 174 | 61 7 | 6 mo | 255 | 77 | (+) | 0 79 |
| 40 | o | 63 | 188 | 85 5 | 2 mo | .00 | 12 | 0 | 1 33 |
| 41 | ♂ | 47 | 170 | 80 5 | 2 mo | 240 | 33 | 0 | 1 56 |
| 42 | o | 56 | 182 | 91 0 | 10 mo | 165 | 64 | 0 | 0 86 |
| 43 | ♂ | 60 | 175 | 64 0 | 6 mo | 270 | 54 | 0 | 1 34 |
| 44 | ♂ | 30 | 188 | 75 0 | 5 weeks | 150 | 3 | 0 | 0 80 |
| 45 | ♀ | 24 | 172 | 69 0 | 1 mo | 150 | 16 | + | 1 10 |
| 46 | ♂ | 55 | 182 | 94 5 | 6 mo | 196 | 113 | 0 | 1 28 |
| 47 | ♂ | 59 | 184 | 114 0 | No sympt | 225 | 30 | 0 | 0 85 |
| 48 | ♀ | 62 | 157 | 66 8 | 4 mo | 175 | 5 | 0 | 1 22 |
| 49 | ♂ | 42 | 178 | 77 5 | 9 mo | 230 | 52 | 0 | 0 90 |
| 50 | ♀ | 72 | 162 | 65 0 | 2 weeks | 170 | 4 | 0 | 1 50 |
| 51 | o | 61 | 180 | 84 0 | 10 mo | 220 | 50 | 0 | 1 30 |
| 52 | ♂ | 38 | 178 | 87 5 | 2 mo | 165 | 26 | 0 | 0 91 |
| 53 | ♂ | 56 | 170 | 66 0 | 5 mo | 125 | 10 | 0 | 1 18 |
| 54 | ♀ | 57 | 157 | 75 5 | 7 mo | 270 | 105 | 0 | 1 09 |

*n* 30
Mean 1 11 g glycogen/100 g wet weight range 0 77–1 56

morning after 12 to 16 hours of fasting and resting. Treatment with insulin and oral antidiabetic compounds was initiated immediately after the first biopsy and the diet regime was not changed during the period of observation. During insulin treatment, muscle biopsies were repeated, if possible within 24 to 48 hours after initiation of treatment. The third biopsy was usually made on to two weeks later

Muscle samples were taken from the lateral part of the *m quadriceps femo is* using the n edle biopsy technique of Bergstrom (1) The muscle samples were rapidly freed from visible fat and conn cu e tissue and weighed on an electromagnetic Cahn balance The samples, used for glycogen determinations, ranged from 5 to 10 mg wet weight, and duplicate determinations were made according to the method of Hultman (16) The biopsy specimens were weighed, homogenized in cold water and the proteins precipitated with trichloroacetic acid within 5 min after biopsy The TCA-soluble g ycogen was precipitated with ethanol and, after hydrolysis with sulphuric acid, determined as glucose by the o-toluidine method according to Hultman (15) Water and fat contents were determined in separate muscle samples. Determinations of glucose in blood and urine were similarly made using the *o* toluidine method

## RESULTS

In Table IV are presented the values for the glycogen content from the various groups of diabetic patients together with those of the control groups The statistical significance of the differences between the mean values for the groups is also given It can be seen that the values for the controls are practically identical with those given by Hultman and do not differ significantly the mean values in each group were 1 40 g/100 g and 1 39 g/100 g The average glycogen value for the untreated juvenile diabetics was remarkably low being 0 49 g/100 g wet weight and in none of these cases did a value occur within the range of the corresponding control values In the insulin-

Table IV *Comparison between the muscle glycogen values of the controls and the basal values of untreated diabetic patients*

| | *n* | Age | *Muscle glycogen g 100 g wet weight* | |
|---|---|---|---|---|
| | | | Mean | Range |
| Normals (Hultman 1967) | 228 | 18–55 | 1 39 | 0 9–2.49 |
| Hospitalized non-diabetics | | | | |
| A Juveniles | 10 | 3–15 | 1 40±0 125 | 1 0 –2 15 |
| B Adults | 16 | 39–78 | 1 40±0 072 | 1 01–2 12 |
| Diabetics | | | | |
| 1 Juveniles insulin-dependent | 12 | 2–15 | 0 49±0 053 | 0 18–0 84 |
| 2 Adults insulin-dependent | 12 | 20–72 | 0 78±0 056 | 0 36–1 06 |
| 3 Adults non-insulin-dependent | 30 | 24–73 | 1 11±0 038 | 0 77–1 56 |

*Statistical comparison between the glycogen values of the different groups*
1 <A ($p<0\ 001$) 2 ($p<0\ 01$) <3 ($p<0\ 001$)
2 <B ($p<0\ 005$) 3 ($p<0\ 001$)
3 <B ($p<0\ 001$)

dependent adults there was again a significant reduction in the level of glycogen the mean value being 0 78 g/100 g wet weight, and only in two cases did the glycogen values come within the limiting values for the corresponding control group The glycogen values for the patients with mild diabetes (group 3) were considerably higher (mean value 1 11 g 100 g) though again still significantly below the normal values However only in seven of the thirty cases examined in this group were the glycogen values below 0 95 g 100 g wet weight and therefore clearly recognizable as pathological

The glycogen values of the diabetic patients

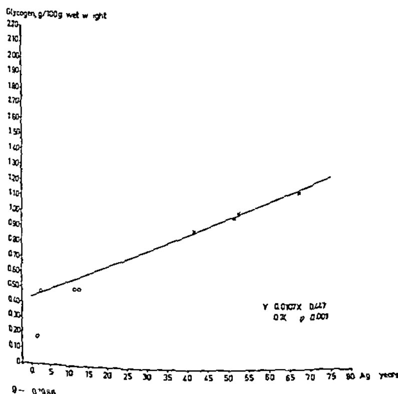



*Fig 1* The muscle glycogen of untreated diabetic patients of various ages. O juvenile diabetes ● insulin-dependent adult diabetes × non-insulin-dependent diabetes.

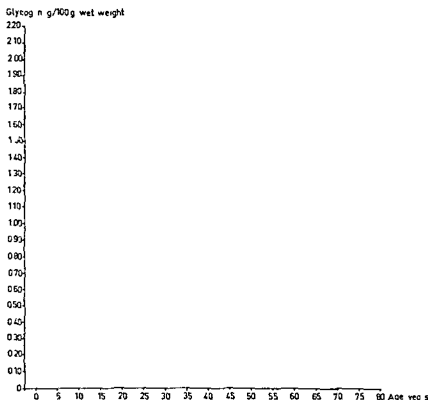



*Fig 2* The muscle glycogen values of 26 hospitalized non-diabetic patients of various ages.

prior to treatment were also related to their ages at the clinical debut of the disease and it appeared that the youngest patients had the lowest glycogen values (Fig 1) In the control material no such correlation was found (Fig 2)

The effect of insulin treatment on the muscle glycogen is shown in Figs 3 and 4 Insulin caused

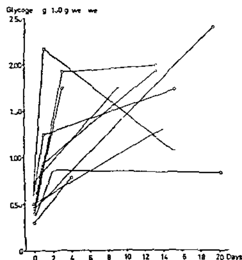



*Fig 3* Juvenile diabetes. The effect of insulin treatment on the muscle glycogen concentration.

a rapid increase in the glycogen of both groups 1 and 2 which was most pronounced in the first few days of treatment The increase in the glycogen of six of the juvenile patients from whom biopsies were taken within one to three days after initiation of insulin treatment was between 0 39 and 1 67 g/100 g wet weight (mean 1 00 g/100 g wet weight) During the corresponding period the glycogen content in six of the adults dependent on insulin treatment increased by 0 08 to 0 61 g/100 g wet weight (mean 0 39 g/100 g) In one case (no 53 Table III) only a slight increase in glycogen content occurred in the first 24 hours though after a week s treatment it had risen from 0 98 to 1 46 g/100 g wet weight In one of the juvenile patients a 12 year old girl (no 4 Table I) who was very nervous motorily restless and finicky about her food there was an initial increase within the first two days of treatment from 0 34 to 0 86 g/100 g wet weight but this did not increase further even after a total of three weeks treatment

For psychological reasons muscle biopsies were not repeated in two of the juvenile cases (nos 11 and 12 Table I) and in one of the adult cases (no 21 Table II) Four of the insulin-dependent

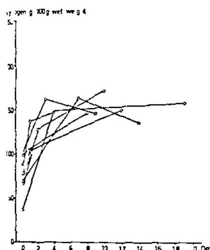


*Fig 4* Adult insulin-dependent diabetes The effect of insulin treatment on the muscle glycog n concentration.

adult patients (Table II) were undergoing further examinations at the time of their treatment and therefore only their basal values are presented

A comparative investigation on the effects of various oral anti diabetic drugs on glycogen metabolism will be presented in a further paper

## DISCUSSION

Recently a series of studies on the metabolism of glycogen in muscle (quadriceps femoris) of normal healthy beings was conducted in our laboratory (3 16 17) using the same needle biopsy technique and methods of analysis as presented in this paper It was found that in 96 % of the subjects examined the glycogen levels varied from 0 95 to 2 0 g/ 100 g wet weight, and variations in the glycogen level of a single individual during a period of five hours of rest and fasting were insignificant. Similarly when the subject was on a normal diet and performing light work only slight variations in the levels of glycogen were noted

Because of the limited age distribution of the subjects presented in Hultman s original survey hospitalized patients lacking symptoms of any metabolic disorders and from the ages of three to seventy three years were examined No divergence was found between the glycogen values of these patients and the normal values previously recorded It would therefore app ar that neither hospitalization nor the age of the subject (from three to seventy three years old) was of importance in determining the muscle glycogen levels

Further studies have shown that medium hard to hard work reduces the glycogen content locally in the working muscle and that when the levels of glycogen reach very low limits the ability to carry out hard work ceases (2 3) The supply of glycogen to the working muscle would thus appear to act as a limiting factor with respect to the muscle s capacity to carry out long time work Under conditions of total starvation following depletion of glycogen by hard work resynthesis of glycogen occurs though at a very low rate A similar effect was noticed when subjects were re fed on a diet devoid of carbohydrates and consisting of fat and protein resynthesis was very slow normal glycogen values not b ing reached until more than a week later Re feeding with a diet rich in carbohydrate following glycogen depletion was however found to greatly stimulate glycogen synthesis and the final levels of glycogen reached under these conditions were far in excess of the normal basal values (2 17)

A moderate decrease in muscle glycogen can also be attained in healthy individuals by total starvation or by feeding on a diet poor in carbohydrates consisting mainly of fat and protein for a period of several days (17) When normal healthy subjects are fed a diet rich in carbohydrates without a preceding glycogen depleting exercise the reverse effect occurs resulting in a moderate increase in muscle glycogen levels

In many respects the effects of diabetes mellitus resemble those of starvation or those produced by a diet deficient in carbohydrates In diabetes mellitus the patient has a much reduced rate of carbohydrate utilization resulting in low levels of muscle glycogen and increased formation of ketone bodies High levels of blood glucose are also found in diabetes These effects are promptly corrected by insulin treatment The low glycogen values which occur in diabetes could result from a lack of carbohydrates to the muscle cells even in the presence of plasma insulin the influx of carbohydrates into the muscle cells being dependent on the peripheral efficacy of insulin By increasing the relative levels of insulin as during insulin administration the influx of carbohydrates is increased and the effects reminiscent of starvation are removed

Over the last decade several workers have no-

ticed that the basal levels of plasma insulin in subjects with diabetes mellitus can be as high as those found in normal subjects and in some cases higher though the insulin response to a glucose load is mostly either delayed or incomplete (6 13 26) In classical juvenile diabetes low plasma insulin levels are nearly always found and no increase occurs following glucose administration (6) By following the response of plasma insulin to a glucose infusion as recommended by many authors as a means of detecting carbohydrate disorders of this type abnormal patterns are obtained in patients with overt or latent diabetes and with prediabetes As an instance of the last condition abnormalities have been noticed to occur in the nondiabetic member of monozygotic twin pairs the other being diabetic (7 9) According to the same authors diabetic like" insulin response curves were also found in 20 % of a population examined, none of whom had any demonstrable carbohydrate disorders (8) From these studies it would appear that the abnormal response of insulin to a high glucose level cannot alone be responsible for the deficiency in carbohydrate metabolism of diabetics and that further factors are involved.

In the present study we have been able to show that in the cases of diabetes examined the glycogen in the muscle is decreased and that insulin ad 'ration restores them to normal values These observations would seem to indicate that a reduction in the sensitivity of skeletal muscle to endogenous insulin could occur during the manifestation of previously latent diabetes. A second possibility in individuals with a previously low insulin response but without any disturbances in their carbohydrate metabolism is that an adaptation to low serum levels occurs in the periphery The breakdown in this adaptive mechanism could then lead to the manifestation of diabetes

The mechanism by which insulin is able to control glycogen and carbohydrate metabolism is still unknown though possible modes for the action of insulin could be the control of membrane permeability to glucose or the regulation of the intracellular metabolism either by moderating the activity of endogenous enzymes or by the induction of enzyme synthesis

As mentioned above muscular work may decrease the muscle glycogen content (3) However in healthy subjects a rapid resynthesis of the glycogen takes place during adequate feeding after glycogen reduction (17) In diabetic children if not in precoma who tend to be very lively compared to adult patients it must ultimately be their reduced ability to resynthetize glycogen which results in their extremely low glycogen values.

The estimation of the severity of the disease in the patients was made empirically from the beginning according to their clinical record The rapid progression of the disease characterized by a severe loss in weight polyuria thirst and the occurrence of ketonuria generally indicated insulin treatment. In every case treatment was started before muscle values were known In all the diabetic patients examined irrespective of their clinical condition a significant decrease in muscle glycogen was found This together with the relationship found to exist between the glycogen values and the ages of the patients when diabetes became manifest indicates that the glycogen content of the untreated patients is related to the severity of the disease

## ACKNOWLEDGEMENTS

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## REFERENCES

1 Bergstrom, J Muscle electrolytes in man. Determined by neutron activation analysis on needle biopsy specimens. A study on normal subjects, kidney patients, and patients with chronic diarrhoea Scand. J clin Lab Invest, Suppl 68 1962.
2. Bergstrom, J & Hultman, E. Muscle glycogen synthesis after exercise An enhancing factor localized to the muscle cells in man. Nature (Lond) 210 309 1966
3 Bergstrom, J & Hultman, E. A study of the glycogen metabolism during exercise in man. Scand J clin Lab Invest. 19 218 1967
4 Bergstrom, J., Hultman, E. & Roch Norlund, A. E. Muscle glycogen in juvenile diabetes before and during treatment with insulin. Nature (Lond.) 198 97 1963
5 Beringer A. Experimentale und klinische Untersuchungen beim menschlichen Diabetes mit Biguaniden. Internationales Biguanid Symposium, p 49 Thieme Stuttgart 1960
6. Berson, S A. & Yalow R. S Immunoassay of plasma

insulin. Ciba Foundation, IVth Colloquia on Endocrinology p. 182. Churchill, London 196..

7 Cerasi, E. & Luft, R. The plasma in ulin response to glucose infusion in healthy subjects and in diabetes mellitus. Acta endocr (Kbh.) 55 278 1967

8. Cerasi, E. & Luft, R.. Further studies on healthy subjects with low and high insulin response to glucose infusion. Acta endocr (Kbh.) 55 305 1967

9 Cerasi, E. & Luft, R.. Insulin response to glucose infusion in diabetic and non diabetic monozygotic t in pairs. Genetic control of insulin response Acta endocr (Kbh) 55 330 1967

10 Fisher N F & Lackey R. W.. The glycogen content of the heart, liver and muscles of normal and diabetic dogs. Amer J Physiol. 7.. 43 19 5

11. Gemmill, C. L. The effect of insulin on the glycogen content of isolated muscles. Bull. Johns Hopk. Hosp 66 23., 1940

1.. Gueness, S. G., Makarewich-Galpern, L. M & Lshenko, S. N Effect of butamide cyclamide chlor cyclamide and chlorpropamide on the glycogen content in various tissues. Vop med. Khim. 6 469 1960

13 Hales, C. N., Plasma insulin in diabetes. Ciba Foundation, Vth Colloquia on Endocrinology p 140 Churchill, London 1964

14 Hildes, J A., Sherlock, S & Walshe, V Liver and muscle glycogen in normal subjects, in diabetes mellitus and in acute hepatitis. I. Under basal conditions. Clin. Sci. 7 .87 1949

15 Hultman, E. Rapid specific method for determination of *aldosaccharides in body fluids. Nature (Lond.)* 183 108 19 9

16. Hultman, E. Muscle glycogen in man determined in needle biopsy specimens. Method and normal values. Scand. J clin. Lab Invest. 19 209 1967

17 Hultman, E. & Bergstrom, J.. Muscle glycogen synthesis in relation to diet studied in normal subjects. Acta med. scand 18.. 109 1967

18 Krahl, M E.. The effect of insulin and pituitary hormones on glucose uptake in muscle Ann. N Y Acad. Sci. 54 649 1951

19 Lackey R. W., Bunde C. A., Gill, A. J & Harris, L. C. Glycogen in alloxan-treated rats. Proc. Soc. Exp Biol. (N Y) 57 191 1944

.0 Larner J Villar Palasi, C & Richman, D J Insulin stimulated glycogen formation in rat diaphragm, levels of tissu. intermediates in short-time experiments. Arch. Biochem. 86 6 1960

1 von Mering, J & Minkowski, O Diabetes mellitus nach Pankreasexstirpation. Naunyn Schmiedeberg's Arch. exp Path. Pharmak. 26 371 1890

2.. Moody A. J & Felber J P.. Effect of insulin on the formation of glycogen by the mouse diaphragm in the presence and absence of glucose Diabetes 15 49.. 1966

23 Vaccarato, R., Rizzo A., Previato G., Mioni, G., Bazzato G & Riz, G The muscle in diabetes mellitus a histologic *(optical and electron microscope)* and biochemical study by means of needle biopsy Europ Ass. for the Study of Diabetes. Fourth Ann. Meeting, Louvain, Belgium July 22- 4 1968

4 Stadie, W C., Haugaard, N & Vaughan, M Quantitative relation between insulin and its biological activity J biol Chem. 200 45 1953

.5 Stadie W C & Zapp J A., Jr The effect of insulin upon the synthesis of glycogen by rat diaphragm in vitro *J biol Chem. 1 0 55 1947*

.6. Yalow R. S & Berson, S A. Plasma insulin concentrations in non diabetic and early diabetic subjects. Determination by a new sensitive immuno-assay technique Diabetes 9 254 1960

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# THERAPEUTIC FASTING

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*Abstract* Twenty obese patients were studied during total fast or on a diet of about 00 calories. The weight reduction was almost the same during the different periods. It was easier for the patients to eat 100 g lean meat a day than to receive no calories at all In none of the cases was hunger any problem. Complications in the form of oedema, weakness, hair loss, and in one case polyneuritis were observed during total fast but never when small amounts of protein were given. Repeated analyses were made of the ketone bodies in blood and urine breath acetone plasma free fatty acids, plasma triglycerides, plasma cholesterol blood lactate blood glucose, serum iron, total iron binding capacity serum urate plasma proteins, and electrolytes in serum and urine

Total fasting" has become an increasingly common method of reducing weight in obese patients (1 3 8 30) Although the history of starvation is as old as mankind and fasting has been practised for thousands of years for religious and ritual as well as hunting purposes little clinical knowledge is so far available as to the advantage of total fasting versus some very caloric deficient intake and to what extent electrolytes should be given (5 6 21)

We have studied 20 patients treated at the Medical Department A for 14–110 days with fasting. Most of them were on total fast only receiving non-caloric fluids ad libitum and vitamins The rest of the patients received 0 5 l of skim milk or 100 g lean meat daily Table I gives the pertinent data of the patients Most of the starved had overt obesity a few of the young women were more concerned with their appearance than their actual weight The weight was reduced in one mildly obese patient with asthma in order to see whether the reduced work load on the lungs would improve the state of the patient which it did not

Particular interest was paid to the ketosis of starvation one of the aspects being to see whether the completeness of the fast could be assessed by repeated measurements of one of the ketone bodies

## METHODS

Acetone was measured by gas chromatography according to Rooth and Östenson (25) 3 hydroxybutyrate (3 HB) and acetoacetate (AcAc) enzymatically (3 hydroxybutyrate dehydrogenase supplied by AB Kabi Stockholm) according to Williamson et al (32) Free fatty acids (FFA) were analysed according to Laurell and Tibbling ( 0) triglycerides by the method of Laurell (19) and lactate enzymatically with the Boehringer system The acid base and electrolyte and urate measurements were performed as part of the clinical routine by the Department of Clinical Chemistry

## CLINICAL RESULTS

The weight loss during and after the stay in hospital is seen in Table I After leaving the hospital two patients gained weight markedly (nos 9 and 10) Disregarding the patients with other diseases and those becoming pregnant after the treatment the weight gain was small in four patients and eight lost additional weight The mean observation time after discharge was 38 weeks

The mean weight loss during the stay in hospital is shown in Fig 1 It will be seen that in one month on 0 5 l of skim milk or 100 g lean meat the patients lost almost as much weight as during total starvation

A typical weight curve is shown in Fig 2 In spite of meticulous total fasting it was found that from the 20th day occasional weight increases occurred due to reduced urinary volumes during preceding days When after total starvation the

Table I *Clinical data of the patients*

| Pat no | Age | Sex | Height (cm) | Overweight at admission ( ) | Weight At admission (kg) | Weight At discharge (kg) | Weight At follow up (kg) | Hospitalization (weeks) | Follow up (weeks) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 51 | ♀ | 160 | 120 | 129 9 | 109 8 | 114 0 | 7 | 65 |
| 2 | 60 | ♀ | 158 | 102 | 116 8 | 98 4 | 93 5 | 7 | 58 |
| 3 | 36 | ♀ | 172 | 55 | 104 4 | 89 7 | 98 2 | 5 | 53 |
| 4 | 26 | ♀ | 159 | 82 | 106 5 | 84 1 | 99 0 | 12 | 46 |
| 5 | 41 | ♀ | 172 | 62 | 109 0 | 84 3 | 77 5 | 17 | 43 |
| 6 | 42 | ♀ | 169 | 143 | 158 2 | 134 0 | 137 0 | 9 | 46 |
| 7 | 29 | ♀ | 170 | 52 | 101 0 | 69 9 | 67 5 | 13 | 43 |
| 8 | 47 | ♀ | 172 | 61 | 108 5 | 91 1 | 94 0 | 5 | 42 |
| 9 | 19 | ♀ | 157 | 40 | 80 2 | 73 0 | 89 0 | 2 | 35 |
| 10 | 55 | ♂ | 175 | 52 | 105 5 | 90 0 | 102 0 | 6 | 42 |
| 11 | 60 | ♀ | 163 | 66 | 101 6 | 90 4 | | 4 | |
| 12 | 76 | ♀ | 153 | 107 | 112 5 | 99 6 | 102 0 | 5 | 37 |
| 13 | 27 | ♀ | 163 | 82 | 111 6 | 86 5 | 77 0 | 8 | 39 |
| 14 | 37 | ♂ | 175 | 62 | 113 7 | 92 6 | 103 8 | 7 | 31 |
| 15 | 39 | ♀ | 157 | 145 | 140 1 | 129 0 | 105 8 | 5 | 19 |
| 16 | 47 | ♀ | 163 | 35 | 82 0 | 6[illegible] 3 | 68 0 | 10 | 26 |
| 17 | 54 | ♀ | 172 | 65 | 111 0 | 99 5 | 85 7 | 5 | 25 |
| 18 | 30 | ♂ | 181 | 100 | 149 7 | 111 0 | 86 8 | 13 | 23 |
| 19 | 27 | ♂ | 184 | 42 | 109 4 | 94 7 | 82 5 | 4 | 20 |
| 20 | 20 | ♀ | 155 | 40 | 77 1 | 68 5 | 69 0 | 3 | 23 |

Complications pats no 2 13 16 and 17 had hair loss no 5 felt ill and no 7 had polyneuritis

patients were given food and usually they were at first only given 100–200 g of lean meat they invariably gained weight and developed manifest oedema which responded to saluretics

One of the patients no 18 had a classical picture of Pickwickian syndrome He fell asleep during classes although he was a teacher A couple of weeks after onset of starvation this disappeared entirely

Patient no 4 had had no menstruation for 20 months prior to admission After seven weeks of fasting her periods returned She was one of the two patients who became pregnant in the follow up period As expected the patients gained weight during their pregnancy and were at least for some time beset with a ferocious hunger

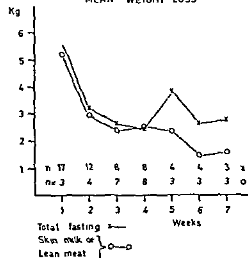



*Fig 1* Mean weight loss per week during the stay in hospital.

### *Side effects*

During or soon after the therapeutic fasting at least four of the patients lost hair Once they began to eat again even if they continued to lose weight their hair began to grow normally again

Only one of the patients no 5 felt really ill during the fast As she showed more metabolic acidosis and a higher degree of ketosis than otherwise seen her total fast was discontinued For further details see Fig 10

Patient no 7 felt a weakness in her limbs on the day of discharge but did not at that time mention it During the following days she rapidly developed a picture of polyneuritis mainly in the legs but also in the arms with considerable difficulty in walking because of bilateral paralysis of

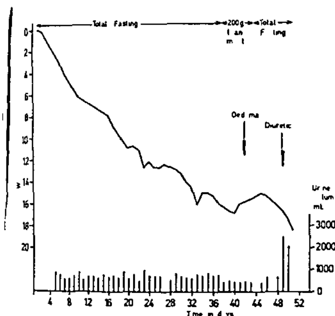



*Fig 2* Weight loss in patient no

the peroneal nerves After a few days she began to improve and two weeks after discharge there were no residual symptoms

## LABORATORY RESULTS

The rate of the increase as well as the maximum level of ketosis varied somewhat but the overall pattern of changes was consistent from case to case Our data agreed with the assumption that the maximal concentration of AcAc + 3 HB observed is inversely related to the degree of overweight. No correlation could be observed between

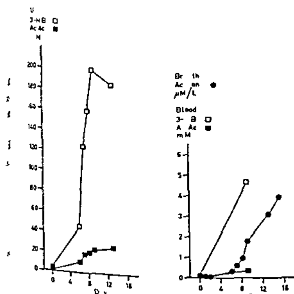



*Fig 3* Onset of ketosis in patient no 15

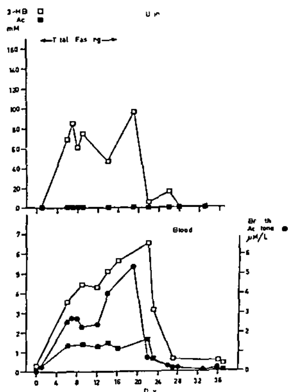



*Fig 4* The appearance and disappearance of ketosis in patient no 10

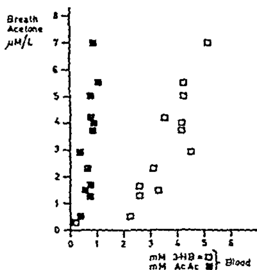



*Fig 5* The relationship between breath acetone and blood keto-acids in patient no 6

the degree of overweight and the rate of onset of ketosis A typical case is shown in Fig 3 It will be seen that after starving for one week there was a rapid increase in ketosis and numerically this was most conspicuous in the urinary concentration of 3 HB Fig. 4 gives the ketosis over a longer period There was a gradual increase in the blood concentration of 3 HB until the patient began to eat when a rapid return occurred to a normal level In the urine there was some variation in the concentration of 3 HB but it remained high as long as the fasting lasted

AcAc increased in the blood during starvation but seldom to a level above 1 mmole/l It is noteworthy that some patients although their blood AcAc level was increased did *not* excrete increased amounts of AcAc This is exemplified in Fig 4 in contrast to Fig. 3

The relationship between the breath acetone and blood 3 HB and AcAc in one representative case is shown in Fig 5 Both fit best with a semilogarithmic line (for AcAc $r=0.92$ and for 3 HB $r=0.80$)

The relationship between the blood and urine concentrations of 3 HB is shown in Fig 6 and again fits best with a semilogarithmic line ($r=0.71$) According to Galvin et al (9) there is a linear relationship between the urinary excretion of AcAc and the plasma level whereas there is a semilog relationship between corresponding 3 HB parameters Our present findings are in agreement with regard to 3 HB but our data indicate that for AcAc (Fig 7) a rather more complex situation may exist as a few of the patients had virtually no AcAc excretion in spite of high blood levels

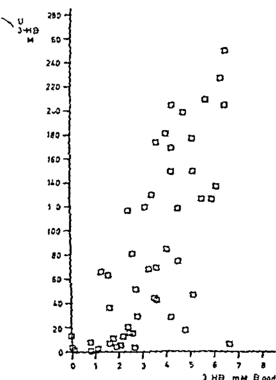



*Fig 6* The relationship between urine and blood concentrations of 3-HB in 15 of the patients.

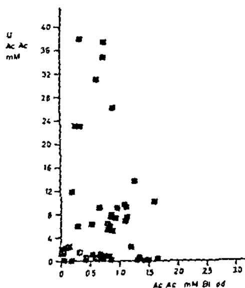



*Fig 7* The relationship between urine and blood concentrations of AcAc in 15 of the patients.

and in the other patients the urinary threshold for AcAc decreased after about ten days of fast

During fasting FFA increased and reached a maximal level of about 1 4 mM as seen in Fig 8

It will be seen from Fig 9 that the relationship between FFA and 3 HB was linear up to about 2 5 mM of 3 HB After that FFA attained its maximal level whereas 3 HB continued to increase

As seen in the lower part of Fig 9 FFA increased in parallel with increases in AcAc as would be expected, as both AcAc and FFA reached a maximal level after some ten days

Lactate was measured in 11 patients before and after a mean of 16 days of fast The blood concentration of lactate increased in all cases by a mean of 0 6±0 18 mEq/l and decreased in the cases studied once the patients began to eat again This increase was highly significant ($P=1/2048$)

Instead of measuring the ketone bodies their effect on the metabolic component of the acid base balance and on pH may be measured as in the ketosis of diabetes The base deficit except for the patient in Fig 10 did not exceed 10 mEq/l She was the only one who became markedly ill during the fasting During this period of ill feeling she not only increased her concentration of ketone bodies to the highest levels observed in this study but developed conspicuous water and electrolyte changes as well and a base deficit of 15 mEq/l She was given food and sodium bicarbonate (2 g × 3 times a day) for four days with a rapid normalization of her general health as well as of her laboratory parameters Her metabolic acidosis was overcompensated but a subsequent renewal of the fasting again led to a rapid increase in the acidosis

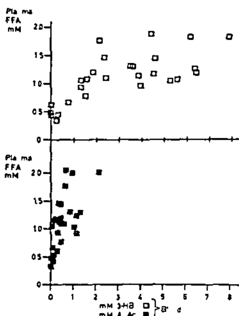



*Fig 9* The relationship between plasma FFA concentration and blood 3 HB and AcAc concentrations respectively in 18 patients.

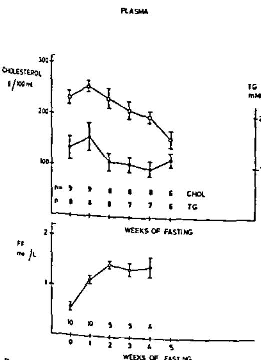



*Fig 8* Plasma FFA, plasma TG and plasma cholesterol concentrations during fasting.

Although there was usually a good correlation between the base deficit and the increase in 3 HB and AcAc as in Fig 10 this correlation could be lost if the patients were given saluretics which produce a metabolic alkalosis or $NaHCO_3$ as just stated

The plasma electrolyte changes are not conspicuous but during total fasting there was a gradual decrease in the plasma potassium and chloride whereas there were no consistent changes in sodium Fig 11

The urinary sodium excretion decreased rapidly as well as that of chloride during total fasting By contrast the potassium elimination was maintained at 40–50 mEq/24 h in most cases for many days

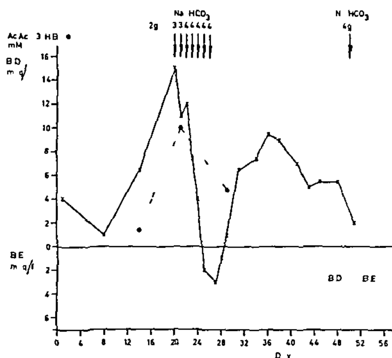



*Fig 10* Variations in the metabolic component of the acid base balance (base deficit or base excess) and blood keto-acids in patient no 5

Table II shows the decrease in serum iron and total iron binding capacity (TIBC) The blood glucose concentration fell in all patients but one (Table II) whereas Drenick et al (7) found no decrease in five out of their 11 patients Plasma urate increased in all cases (Table II) The increase was of the same order of magnitude whether the patients were receiving no calories or were getting lean meat or fish or skim milk In five cases plasma protein electrophoresis was performed with about four weeks interval The results in Table II indicate contrary to the results of Drenick et al

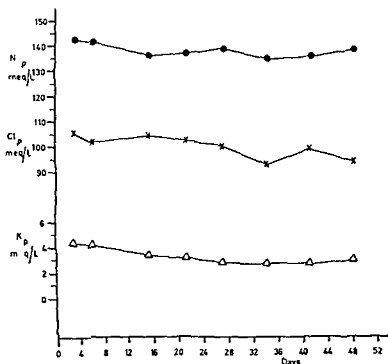



*Fig 11* Plasma electrolytes in patient no 6

decrease in the protein fractions. This was not qually marked in all fractions indicating that aemodilution was only one of the reasons for his decrease

## DISCUSSION

uring starvation triglycerides in adipose tissue re broken down and the liberated fatty acids are obilized from adipose tissue to plasma This ads to an elevation of the plasma free fatty acid ncentration. An important part of the fatty acids e then taken up by the peripheral tissues and etabolized serving as fuel for muscular work d other metabolic purposes Another part of e fatty acids is metabolized in the liver The dif rent metabolic pathways in the liver cell are hematically outlined in Fig 12 During total st the majority of the fatty acids are metabolized the liver as the mobilization of fatty acids om adipose tissue exceeds the energy require nt of the peripheral tissue.

It is apparent from Fig. 12 that the fatty acids the liver initially are converted to acetyl co- zyme A (AcCoA) which is a key substance in er metabolism AcCoA is then further metabo- d mainly along three pathways 1) it may be d for synthesis of for instance triglycerides

ble II *Some mean laboratory values before and after ting for about four weeks*

| | Before | After | No | Significance level of individual diff |
|---|---|---|---|---|
| s (g 100 ml) | 138 8 | 74 1 | 12 | $P<0\ 001$ |
| C,s (g 100 ml) | 351 8 | 270 3 | 14 | $P<0\ 001$ |
| d glucose (g 100 ml) | 83 6 | 63 3 | 15 | $P<0\ 001$ |
| te s (g 100 ml) | 5 5 | 11 2 | 16 | $P<0\ 001$ |
| aman s 100 ml) | 4 68 | 4 50 | 5 | |
| bulins 100 ml) | | | | |
| | 0 32 | 0 30 | 5 | |
| | 0 57 | 0 48 | 5 | |
| | 0 47 | 0 34 | 5 | |
| | 0 29 | 0 22 | 5 | |
| | 1 06 | 0 93 | 5 | |
| d proteins 100 ml) | 7 42 | 6 84 | 5 | |

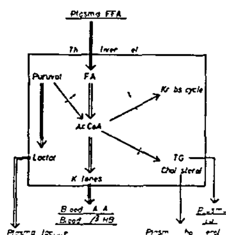



*Fig 1* Schematic representation of the liver metabolism of the fatty acids. The dotted lines represent partially blocked reactions. The underlined plasma metabolites were measured in the present study

and cholesterol which substances may escape from the liver and form part of the plasma tri glycerides and plasma cholesterol content 2) it may be burnt in the Krebs cycle 3) it may be transformed to ketone bodies

During fasting when the supply of glucose is restricted the first and the second pathways are partially blocked and the majority of the AcCoA formed is therefore converted into ketone bodies These are therefore produced in excess AcAc and 3 HB pass into the plasma and a state of metabolic acidosis ensues

AcCoA is usually also formed from pyruvate in the liver but due to the increased formation of AcCoA from fatty acids during fasting this process is partially blocked too and increased amounts of lactate are produced instead This in turn results in an elevation of the blood pyruvate and lactate levels. Voigt and Apostolakis (31) found increased blood pyruvate level and the present lactate results are also in agreement with this conception

As is well known the plasma FFA level rises during fasting and we found about double the initial level after two weeks of fasting which is in agreement with earlier findings (2 18 24)

Returning to Fig. 12 the increase in the ketone bodies is considerable but only after a few days of fast in these obese patients See also Kekwick et al (17) and van Itallie and Bergen (15) Most of the ketone bodies produced in

used for metabolic purposes and only minor amounts are eliminated via the respiration and the urine

During prolonged fasting the haemoglobin concentration as well as the serum iron concentration fell as also found by Thomson et al (30) The decrease in the total iron binding capacity is in agreement with the findings of McFarlane et al (22) who found TIBC to be the best biochemical assessment of protein caloric malnutrition Jagenburg and Svanborg (16) observed one patient with prolonged starvation During starvation his TIBC was 170 $\mu$g/100 ml and increased to 435 after refeeding

Another symptom of protein lack is a change in the hair roots (4) The hair loss observed in some of our patients could perhaps have justified a study of the hair roots as has been done in the protein deficient South American Indians of the Peruvian Andes

From our present experience total fasting for longer periods is not advisable as we observed unwanted side effects hair loss weight increase and oedema muscular weakness and in one case polyneuritis Other complications have been described too Drenick et al (7) saw arterial hypotension Norbury (23) anuria with death Spencer (28) death due to heart failure in patients given diu[illegible] Garnett et al (10) observed one otherwise [illegible]althy young woman who died during refeeding At autopsy there were signs of myocardial atrophy

If the patients are given electrolytes (5 6) they fare better In order to give some electrolytes and to reduce the endogenous protein breakdown we gave skim milk or 100–200 g of lean meat to some of the patients in the present study and to other patients treated subsequently and observed no complications and no oedema. They felt well and were able to work (including reading for examinations and other intellectual activities) The patients on skim milk or lean meat or fish have lost appetite just as rapidly as those on total fasting

The normal breath concentration of acetone is $<0.02$ $\mu$moles/l alveolar air Increasing several hundred times as it does during starvation and being measured in less than one minute it is the ideal parameter to check the completeness of the patient s fast. It was constantly observed that when the patients broke fast for one reason or another 3 HB in blood and urine decreased as well as breath acetone The patients learned that they could not cheat and therefore at once fasted as ordered

Another advantage of the acetone measurements was that the breath acetone concentration increased or remained elevated during fasting regardless of the body weight changes The fasting patients became depressed when they increased in weight but were encouraged when judging from the acetone level we could tell them that in spite of a weight increase they were losing as much fat as before

The mechanisms of the water changes have been studied by Schloeder and Stinebauch (26) by Stinebauch and Schloeder (29) and by Garnett et al (11)

Diuretics are not infrequently given to obese fasting patients This would seem to be an error The fasting in itself whether total or of the skim milk type promotes considerable water loss during the first week due to the acidosis By giving diuretics at the onset of starvation the water loss becomes extreme and the electrolyte disturbances will be more pronounced Two of the patients in other studies who died during total fasting had been given diuretics (28)

Judging from the experience with diabetes it would be expected that the level of ketosis and consequently the adherence to the starvation programme can be followed from measurements of the acid–base balance However this may be fallacious We observed no systematic pH changes in accordance with Schmidt et al (27) or Voigt and Apostolakis (31) as $pCO_2$ falls with increasing base deficit On the whole there is a stoichiometrical relationship between the total increase in keto acids and base deficit but if the patients are given saluretics or sodium bicarbonate the metabolic acidosis apparently disappears i e the base deficit decreases and a base excess may develop whereas the concentration of keto acids remains unchanged

Again Acetest made on urine specimens is not a reliable method for assessing the fast as some patients do not excrete AcAc in the urine in spite of increased blood levels

Most authors are pessimistic about the long term results of fasting (13 14) Even if this pessimism were justifiable there is no doubt that some patients are so incapacitated by their obesity that even a temporary weight reduction is de

urable. In the present series the patients have remained at their weight on discharge or have co tinued to lose weight in most cases It is our feeling that one of the reasons why the present long term results have been better than some published is that the patients lost so much weight that they were much leaner after discharge This has had a great psychological effect particularly on several of the young people Having had to buy new clothes they were stimulated to remain within the newer narrower limits of their waist bands

Patient no 18 with the classical Pickwickian syndrome was at admission completely bald al though only 30 years of age During the follow up period his hair has began to grow again Patient no 4 had very little hair on her head but after fasting for ten days her hair also began to grow again The reappearance of hair growth during or after starvation in two of the patients may per haps be attributed to endocrine changes parallel to the reappearance of periods in the woman

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## REFERENCES

1 Asfeldt, W H., Grønbæk, P & Iversen M Uge kr Læg. 1 7 983 1965
2 Bloom, W L Azar G & Clark J E. Metabolism 15 401 1966
3 Bloom, W L. & Mitchell W Arch. intern Med 106 321 1960
4 Bradfield R. B Bailey M A & Cordano A. Lancet 2. 1169 1968
5 Consolazio C F Matoush L. O., Johnson, H L. Kryzwicki, H. J Isaac G J & Witt N F Amer J clin. Nutr 21 793 1968
6 — Amer J clin. Nutr 21 803 1968
7 Drenick E. J Swendseid M E Bladh W H & Tuttle S G J Amer med. Ass. 187 100 1964
8 Duncan, L. E Liddle C W & Bartler F C J clin. Invest. 35 1299 1956
9 Gal in, R. D., Harris, J A. & Johnson, R. E. Quart. J exp Physiol 53 181 1968
10 Garnett E S Barnard D L Ford, J Goodbody R. A. & Woodehouse M A. Lancet 1 914 1969
11 Garnett, E. S Ford, J Golding, P L., Mardell R. J & Whyman, A. E Clin Sci 35 93 1968
12 Hansen, E. L Hørlyck, E Grønbæk, P & Iversen, M Acta med scand. 182 65 1967
13 Harrison M T & Harden R. McG Lancet 2 1340 1966
14 Hermann L S & Iversen, M Lancet . 217 1968
15 van Itallie T B & Bergen S S Jr Amer J Med. 31 909 1961
16 Jagenburg, R. & Svanborg, A. Acta med scand. 183 67 1968
17 Kekwick A Pawan G L S & Chalmers T M Lancet 2 1157 1959
18 Laurell S Scand J clin. Lab Invest 8 81 1956
19 — Scand. J clin Lab Invest 18 668 1966
20 Laurell S & Tibbling, G Clin chim Acta 16 57 1967
21 Maagoe H Metabolism 17 133 1968
22 McFarlane H Ogbeide M I Reddy S Ad o I K. J Adeshina, H Burney J M Cooke A Tay lor G O & Mordie J A Lancet 1 39 1969
23 Norbury F B J Amer med Ass. 188 88 1964
24 Persson, B E H & Sterk G C G Acta paediat. (Uppsala) 55 153 1966
25 Rooth G & Östenson S Lancet 2 1107 1966
26 Schloeder F X & Sunebauch B J Metabolism 15 838 1966
27 Schmidt H Janik, I & Voigt K D Dtsch med Wschr 94 78 1969
28 Spencer J O B Lancet 1 1 88 1968
29 Sunebauch, B J & Schloeder F X Met bolism 15 828 1966
30 Thomson, T J Runcie J & Miller V Lance 2 992 1966
31 Voigt, K. D & Apostolakis M Klin Wschr 47 157 1969
32 Williamson D H Mellanby J & Krebs H A Biochem. J 82 90 196.

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# ECG IN STRICTLY POSTERIOR MYOCARDIAL INFARCTION

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*Abstract* ECGs from 215 patients with a diagnosis of recent myocardial infarction and a case history shorter than 48 hours have been examined. Sixty-eight had anterior myocardial infarctions (group 1) Seventy-seven had posterior myocardial infarctions (group 2) Seventy had equivocal ECG signs of recent myocardial infarction (group 3) In group 3 thirteen patients had R/S quotients >1 in V and/or $V_2$. In group 2 sixteen patients had R/S quotients >1 in V and/or V together with the ordinary posterior myocardial infarction pattern. In the absence of right bundle branch block, WPW syndrome congenital heart disease chronic advanced pulmonary disease or any other diseases which cause pure right ventricular hypertrophy this sign points to a myocardial infarction in the strictly posterior (or posterolateral) aspects of the left ventricle The electrophysiological basis of this finding is discussed briefly It is stressed that this ECG sign is not rare as it was the sole ECG sign of recent myocardial infarction in 6 of patients in this study In another 8% it was seen together with the ordinary Q wave pattern of "posterior" (diaphragmatic) myocardial infarction

It is well known that small infarctions and subendocardially located infarctions may be difficult or even impossible to diagnose in the ECG A second or third myocardial infarction in the same patient often also creates insurmountable obstacles to the ECG reader Furthermore it is easy to overlook myocardial infarctions with special locations using conventional scalar 12 lead ECG

Levy et al (13) noted that tall R waves and shallow S-waves occurred or developed in $V_1$–V in a certain number of patients with a fresh myocardial infarct Several authors have later called attention to the same finding which points to a lesion in the strictly posterior aspects of the left ventricle (4 7 8 17 18 19 27) This pattern seems to be unknown to many of those who use strict scalar methods in describing the ECG

Our intention has been to discuss the frequency and importance of this particular ECG pattern in an unselected group of patients with a fresh myocardial infarct

## MATERIAL AND METHODS

ECGs from 215 patients have been examined The 215 patients represent the total number of patients with the diagnosis of a recent myocardial infarction during a period of two years in our department provided that at least two ECGs had been taken (ordinary 12 lead ECG) In addition the case history had to be of less than 48 hours duration before admission to the hospital No patient has been included more than once The diagnosis is based on the case history ECG findings and SGOT values rising to at least 40 Sigma units Beside we have paid attention to the leucocyte counts ESR, the temperature curve and serum LDH in a few doubtful cases

On the basis of the ECG pattern we have made the following subgrouping of the 215 patients

Group 1 patients with anterior myocardial infarctions
Group 2 patients with posterior myocardial infarctions
Group 3 patients whose ECGs do not prove the existence of myocardial infarctions

Pathological Q waves in lead I aVL and or V–V have been taken as proof of an anterior myocardial infarction while pathological Q waves in II III aVF have pointed to a posterior myocardial infarction The same ECG criteria have been used in previous work from our department (2, 3) ECGs without such Q changes have been called atypical All ECGs have been examined with special reference to the R/S quotient in V and V R/S quotients >1 have been particularly noted

## RESULTS

Sixty eight patients had anterior myocardial infarction (group 1) (including anteroseptal strictly anterior anterolateral and apical myocardial infarctions) By definition none in this group had an R/S quotient >1 in $V_1$–V

Seventy seven had a conventional posterior myocardial infarction (group 2) (including diaphragmatic and diaphragmaticolateral myocardial infarctions) In this group (Table II) six had R/S quotients >1 in $V_1$ *and* V while ten had an R/S quotient >1 in V but not in $V_1$ Several had insignificant Q waves in $V_5$–$V_6$ in this group

Table I *Patients with R/S ratio greater than 1 in $V_1$ and/or $V_2$ (without significant Q waves 12 leads)*

| Case no | Age | On admission (mm) $R_V$ | $S_V$ | $R_V$ | $S_V$ | On leaving hospital (mm) $R_V$ | $S_V$ | $R_V$ | $S_V$ | SGOT (max value) | Remarks (case history before adm ) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 58 | 3 | 0 | 6 | 0 | 6 | 0 | 11 | 0 | 127 Sigma u | No Q waves | (18 h) |
| 2 | 62 | 1 5 | 3 5 | 5 | 6 | 1 5 | 0 | 8 | 4 5 | 300 | $Q_{2\ 3-aVF}$ | (5 h) |
| 3 | 59 | 1 5 | 2 | 6 | 4 | 4 | 0 | 8 | 1 5 | 159 | $Q_{3\ aVF}$ | (4 h) |
| 4 | 63 | 6 5 | 0 | 24 | 4 | 4 | 1 | 13 | 3 | 240 | $Q_{1-2\ aVL\ V6}$ | ( 4 h) |
| 5 | 53 | 3 5 | 0 5 | 6 | 7 | 2 | 1 | 8 5 | 8 | 120 | $Q_{V6}$ | (14 h) |
| 6 | 53 | 3 | 8 5 | 6 | 13 | 9 | 0 | 14 5 | 2 | 340 | No Q waves | (3 h) |
| 7 | 59 | 1 5 | 1 5 | 11 | 3 | 3 5 | 1 5 | 12 | 4 | 330 | No Q waves | (25 h) |
| 8 | 46 | 2.5 | 0 5 | 11 | 8 | 2 | 1 | 6 | 1 | 134 | $Q_{2\ -\ VF}$ | (18 h) |
| 9 | 81 | 0 5 | 0 5 | 1 | 0 5 | 0 | 1 | 2 | 1 | 162 | No Q waves | (36 h) |
| 10 | 51 | 1 5 | 6 | 6 | 11 | 2 | 0 | 10 | 5 | 160 | $Q_{2\ 3\ aVF}$ | (2 h) |
| 11 | 68 | 1 5 | 5 | 9 | 5 | 2 5 | 2 5 | 9 | 5 | 107 | $Q_{2\ 3\ aVF}$ | (12 h) |
| 12 | 51 | 0 5 | 4 5 | 5 5 | 4 | 1 5 | 3 | 9 | 3 | 162 | $Q_{1-aVL-V6}$ | (2 h) |
| 13 | 56 | 2 | 3 | 5 | 2 | 1 5 | 1 | 5 | 2 | 127 | $Q_3$ | (10 h) |

Seventy patients had equivocal ECG signs of recent myocardial infarction (group 3) Of these seventy patients fifteen had R/S quotient > 1 in $V_1$ and/or V Two of these fifteen patients had to be excluded because of technical errors in the ECG(s) which left only one ECG to be ex amined thoroughly The thirteen patients are pre sented in Table I As can be seen ten had R/S quotients > 1 in both $V_1$ and V while three had R/S quotients > 1 solely in V Nine in this group ( Table I) had insignificant Q waves in the terior leads (II III aVF) or the anterior ds (I aVL V $-V_6$)

All patients with R/S quotients > 1 in group 2 and group 3 had positive T waves in $V_1$ and V None of them had right axis deviation while abo 50° had left axis deviation

As can be seen from Tables I and II all th patients with the special R/S pattern had high significant SGOT values The lowest value amor the patients from group 2 was 64 Sigma unit while it was 107 Sigma units in group 3

The transaminase values do not differ fro the average values in the total 215 patients (? None of the sixteen patients with R/S quotien > 1 in $V_1$ and/or V from group 2 nor th thirteen patients from group 3 had WPW sy drome congenital heart disease advanced chron

Table II *Patients with diaphragmatic infarction ( posterior ) with R/S ratio greater than 1 in $V_1$ and or V*

| Case no | Age | On admission (mm) $R_V$ | $S_V$ | $R_V$ | $S_V$ | On leaving hospital (mm) $R_V$ | $S_V$ | $R_V$ | $S_V$ | SGOT (max value) | Remarks (case history before adm ) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 61 | 7 5 | 4 | 11 5 | 11 | 3 5 | 2 | 12 | 10 | 400 | $Q_{2\ 3-aVF}$ | (7 h) |
| 2 | 67 | 2 5 | 5 5 | 11 5 | 13 | ? 5 | 4 | 12 | 10 | 127 | $Q_{2\ 3\ aVF}$ | (2 h) |
| 3 | 67 | [illegible] | 11 | 3 5 | 6 5 | 2 | 5 | 6 | [illegible] 5 | 88 | $Q_{2\ 3\ aVF}$ | (3 h) |
| 4 | 63 | 3 | 13 | 6 5 | [illegible]0 | ? | 1 | 7 | 3 | 980 (shock) | $Q_{2\ 3\ aVF}$ $QS_{V4}$ | (4 h) |
| 5 | 57 | 9 | 7 | 7 | 17 | 5 | 6 5 | 9 | 7 | 190 | $Q_{2\ 3-aVF}$ $Q_{V2-V4}$ | (3 h) |
| 6 | 57 | 3 | 6 5 | 10 | 8 | 3 | 6 | 9 | 2 | 370 | $Q_{2\ 3\ aVF}$ | ( h) |
| 7 | 62 | 3 5 | 4 5 | 9 | 7 5 | 4 5 | 5 5 | 11 | 11 | 134 | $Q_{\ 3-\ VF}$ | (8 h) |
| 8 | 60 | 0 5 | 11 | 2 | 11 | 1 | 5 | 8 5 | 5 | 540 | $Q_{\ 3\ aVF}$ earl $QS_{V2\ V}$ | (19 h) |
| 9 | 67 | 3 | 5 | 0 | 11 | 6 5 | 6 | 14 | 7 | 250 | $Q_{\ 3-\ VF}$ decr $R_{V2\ 3-}$ | ( h) |
| 10 | 61 | 1 | 5 | 4 | 0 | 2 | 4 | 6 | 0 | 137 | $Q_{\ 3\ VF}$ | ( h) |
| 11 | 52 | 3 | 11 | 4 | 14 | 3 | 4 | 3 5 | 3 5 | 660 | $Q_{2\ 3\ VF}$ | (2 h) |
| 12 | 47 | 4 | 6 | 1 | 2 | 3 5 | 5 | 16 | 0 | 360 | $Q_{\ -aVF}$ | (1 h) |
| 13 | 65 | 8 | 4 | 13 | ? | 5 | 2 | 14 | 1 | 64 | $Q_{2\ \ VF}$ $Q_{V2-V4}$ | (3 h) |
| 14 | 61 | 0 5 | 12 | 6 | 17 5 | 5 | 8 5 | 7 5 | 5 | 173 | $Q_{\ 3-aVF}$ $Q_{V2-V}$ | (1 h) |
| 15 | 76 | 1 5 | 7 | 2 | 6 | 1 | 5 | [illegible] | 1 | 3-0 | $Q_{2\ 3\ VF}$ $S_V = 0$ | (1 h) |
| 16 | 58 | 12 | 11 | 16 | 17 | 9 5 | 4 | 18 | 9 | ?00 | $Q_{\ 3-\ VF}$ $Q_{V4\ V6}$ | ( 4 h) |

pulmonary disease or any other disease that might have caused pure right ventricular hypertrophy

Neither had any of them right bundle branch block The youngest patient was forty six years old on admission to the hospital Almost all the ECGs to be examined have been taken by the same experienced ECG operator

*The mortality rate in group 3* as a whole was half the total mortality (2)

## DISCUSSION

In myocardial infarction (or rare other forms of heart muscle destruction) (20) the QRS complex is generated from areas with normal heart muscle ie from areas more or less opposite to the in farction We shall then usually find pathological Q-waves in one or more of our conventional leads When such Q waves are lacking one usually speaks of atypical ECG

In first definite myocardial infarction ECG is said to be diagnostic in from 70 (21) to 90 (5) of the cases In a previous study from the same department an 80 score of diagnostic changes was found using the same criteria as have been used in this work (3)

Most authors have now abandoned the electri cal window theory" of Wilson for various reasons (10 25) This *theory* cannot explain for ex ample the mirror image changes in the ECG taken from different parts of the chest (Figs 1 and 2) From the vector approach we must assume that areas with positive deflections must have their corresponding areas with exactly the same negative deflections The corresponding areas will be found directly opposite to each other on a diam eter drawn through the electrical center of the heart (Fig 2) A small aberration from the theo retically expected values is seen because the mir ror image points on the back of the chest are at a greater distance from the generator (heart than the corresponding points on the front of the chest

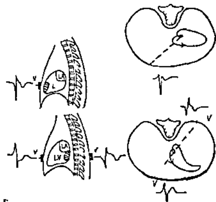



*Fig 1 Upper left* V pattern in anterior myocardial in farction *Lower left* V pattern and minus V pattern in strictly posterior myocardial infarction *Upper right* Normal ECG in V and normal horizontal vector electro cardiogram *Lower right* ECG in V and minus V in strictly posterior *myocardial* infarction and vectorelectro cardiogram in the same condition

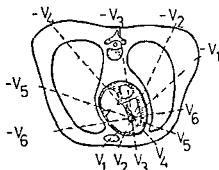



*Fig 2* Precordial leads V -V and their mirror image points on the chest

Theoretically therefore Q waves on the chest will have *their corresponding* R wave and vice versa Pathological R waves in special leads will therefore prove infarction as efficiently as Q waves in other of our conventional leads (9 10)

Strictly posterior myocardial infarction (with or without extension laterally) will create pathologi cal Q waves on the left side of the back (Figs 1 2) with corresponding pathological R waves in $V_1$–V position The R/S changes in $V_1$–V have been stressed by Levy et al (13) in 1950 and later by Jaffe (11) Fisch (7 8) Perloff (17 18 19) and Zakopoulos (27)

Perloff (19) compares the vector and scalar findings in strictly posterior myocardial infarc tion and finds a special value of the following signs in the scalar ECG

1 0 04 R wave in $V_1$ (75 % of cases)
2 0 04 P wave in V (95 of cases)
3 R/S ratio > 1 in $V_1$ (60 % of cases)
4 R/S ratio > 1 in V (100 of cases)
5 Left axis deviation in 50 of the cases to- *gether with one or more* of the four signs above

Some of these findings however could be found in persons judged to have completely normal hearts Criteria 1 and 3 were never found among one hundred normal controls while criteria 2 and 4 were found in a few per cent (19)

Positive T waves were found in 50% of Perloff's patients in $V_1$ and in 20% of his control group (19) With reference to the R/S changes he mentions the following pitfalls

1 Complete right bundle branch block
2 WPW syndrome type A (anterior-directed delta wave)
3 Right ventricular hypertrophy
4 Child age
5 Normal finding in a few adults (rare after the age of forty and then almost only together with a perpendicular or right axis deviation in the frontal plane)

As pitfall no 6 the author proposes the addition of wrongly placed electrodes Placing the $V_1$ electrode to the right tends to eliminate the finding, while the opposite results from placing the $V_1$–$V_2$ electrodes too much to the left

Abildskov and Boyle (1) and Mathur and Kumar (15) have stressed the importance of changes in the later parts of the QRS in myocardial infarction in their experimental work on dogs Na et al (16) adopted the same points of view their work on scalar ECG taken with Frank's corrected orthogonal lead system In their opinion the measurement of Q R ratios (or R S ratios) contributed to a more correct diagnosis of myocardial infarctions than using Q-wave duration and Q-wave height solely Weisbart et al (24) are of the same opinion Reduction of R wave height, therefore may be a most valuable sign together with Q-wave development (or conversely S reduction with R wave development in diametrically located leads) Besides since the posterior aspects of the left ventricle are the last parts to be excited late QRS changes may be of special importance in strictly posterior myocardial infarction. In ordinary 12 lead ECGs this would mean that S reduction *and* R wave increase would be the expected changes in strictly posterior myocardial infarction.

Tranchesi et al. (23) assume that strictly posterior myocardial infarctions are rare In this material thirteen had an R S quotient > 1 in $V_1$ and/or $V_2$ as the sole pathological change In addition to "conventional posterior myocardial infarction" patterns sixteen had R/S changes of the same type in $V_1$–$V_2$ This special pattern is therefore found in 14% of the cases (13 + 16/215)

Sayen et al (22) draw the following conclusion "Perhaps the simplest general summation is to say that the damage in the left ventricle (except for the massive regional infarct) tends to begin elsewhere than in the anterior coronary region." Coexistent occlusive disease in the right coronary artery and left circumflex artery with posterolateral damage was particularly frequent in their work If their statement is of general value we shall expect the aforementioned R/S pattern in $V_1$–$V_2$ to be a relatively frequent finding in myocardial infarction According to the transaminase values in our patients their infarctions were not particularly small which indeed was the fact in Sayen et al s group of patients which came to autopsy (22)

According to pathoanatomical studies (11 26) there is often a considerable discrepancy between the anatomical and electrocardiographic location of the infarction The importance of this finding is discussed in another study from our department (6) Levy et al (13) however noted that in their experience the R/S pattern in $V_1$ almost always pointed to a lesion in the posterolateral aspects of the left ventricle

The "Q area" (10) and extension of an infarction do not show strict correlation Despite that we may assume that the extension of an infarction is underestimated during routine ECG readings if the special R/S pattern in $V_1$–$V_2$ is not considered It may indeed even be easy to overlook an infarction when a conventional 12 lead scalar ECG is used

## CONCLUSION

When the pitfalls which Perloff (19) mentions can be excluded an R/S quotient > 1 in $V_2$ points to an infarction in the strictly posterior aspects of the left ventricle with or without extension to the lateral wall The same changes in V should be viewed with great suspicion especially if the R wave duration is ≥ 0 04 A positive T wave deflection in $V_1$ and a left axis deviation supports the diagnosis.

The typical R S pattern was the sole ECG sign

myocardial infarction in six per cent of our 5 patients. Eight per cent showed the same changes in addition to conventional posterior myocardial infarction pattern It is therefore assumed that this ECG pattern is *not* rare

## REFERENCES


1 Abildskov J A. & Boyle R. S Further studies of the electrocardiographic effects of experimental myocardial lesions. Amer Heart J 69 49 1965

2 Anderssen, N Erikssen, J & Muller C The prophylactic antiarrhythmic effect of quinidine in myocardial infarction. A controlled clinical trial Acta med. scand 184 171 1968

3 Anderssen, N & Skjæggestad Ö The electrocardiogram in patients with previous myocardial infarction Acta med scand 1 6 123 1964

4 Cohen, A. J Joseph S K. & Markowitz, M The electrocardiogram in strictly posterior myocardial infarction. Dis. Chest 48 527 1965

5 Enger E Julsrud A. & Lygren T Diagnosen av akutt hjerteinfarkt spesielt med henblikk på nytten av de unipolare bryst og ekstremitetsavledninger T norske Lægeforen. 80 633 1960

6 Erikssen, J & Anderssen N To be published

7 Fisch, C. Value of anterior chest leads in the diagnosis of the diaphragmatic and posterior myocardial infarction. J Indian med Ass. 54 495 1961

8 — Posterior myocardial infarction J Indian med Ass 58 1146, 1965

9 Freeman Z. The electrocardiographic changes in myocardial infarction Med J Aust 47 487 1960

10 Grant, R. P.. Clinical electrocardiography the spatial vector approach. M Graw Hill, New York 1957

11 Jaffe H. L Typical and atypical electrocardiograms in myocardial infarction caused by a ut coronary insufficiency Dis. Chest 27 243 1955

12 Levine H D & Phillips E Apprai al of the newer electrocardiography correlations in 150 consecutive autopsied cases. New Engl J Med. 245 833 1951

13 Levy L., Jacobs, H. J Chastant, H P & Strauss H. B Prominent R wave and shallow S-wave in lead V a a result of lateral myocardial infarction Amer Heart J 40 447 1950

14 Lipman, B S & Massie E Clinical scalar electrocardiography Fifth ed. Year Book Medical Publ. Chicago 1965

15 Mathur K. S & Kumar V An experimental study of anterior myocardial infarction and correlation of electrocardiographic changes with localisation of infarct. J Indian med Ass 44 56 1965

16 Naval, I A., Cosma, J & Pipberger H. V Re evaluation of the Q wave in the electrocardiographic diagnosis of myocardial infarction Med Ann D C 36 349 1967

17 Perloff, J K. Recognition of strictly posterior (intra atrial) myocardial infarction by conventional scalar electrocardiography II Circulation 24 1014 1961

18 — The electrocardiographic diagnosis of strictly posterior myocardial infarction CP (Kansa ) 9 128 1964

19 — The recognition of strictly posterior myocardial infarction by conventional scalar electrocardiography Circulation 30 706 1964

20 Pruitt R D Dennis E. W & Kinard S A The difficult electrocardiograph diagnosis of myocardial infarction Progr cardiovasc Dis 6 85 1963

21 Rosenberg, B & Malach, M Acute myocardial infarction in a city hospital IV Clinical pathologic correlations Amer J Cardiol. 6 / 19 60

22 Sayen J J Sheldon W F & Wolferth C C The heart muscle and the electrocardiogram n nar disease III A new classification of ventricular myocardial damage derived from the clinicopathologic findings in 100 patients II Circulation 1 68 1955

23 Tranchesi J Teixeira, V Ebaid M Boccalandro I Bocanegra, J & Pileggi F The electrocardiogram in dorsal or posterior myocardial infarction Amer J Cardiol 7 505 1961

24 Weinbart M & Simonson E Diagnostic accuracy of Q and related electrocardiographic items for the detection of patients with posterior wall myocardial infarction Amer Heart J 50 6 19 5

25 Whipple G H Current concept in electrocardiography A critique of the unipolar approach to interpretation Med. Clin N Amer 41 119 9

26 Woods J D Laurie W & Smith G The reliability of the electrocardiogram in myocardial infarction Lancet 2 265 1963

27 Zakopoulos, K. S & Tsatas A T On strictly posterior" myocardial infarction Dis Che 49 54 1966



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# CATECHOLAMINES AND METABOLISM OF HUMAN ADIPOSE TISSUE

## II. *Effect of Isopropylnoradrenaline and Adrenergic Blocking Agents on Lipolysis in Human Omental Adipose Tissue in vitro*

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*Abstract* Human omental tissue was incubated in Krebs-Henseleit bicarbonate medium containing 3% albumin and glucose with additions of noradrenaline, isopropylnoradrenaline or theophylline. Glycerol release was measured and taken as the index of lipolytic activity.

In the first series of experiments the effects of equimolar concentrations of noradrenaline and isopropylnoradrenaline were compared. At each concentration isopropylnoradrenaline produced significantly higher glycerol release. Noradrenaline-stimulated lipolysis was suppressed completely by the beta adrenergic blocking agent propranolol added at a concentration of 1 $\mu$g per ml of the incubation medium. At this concentration propranolol had no effect on basal glycerol release and that induced by theophylline (10[illegible] M). The alpha adrenergic blocking agent phentolamine inhibited only in some experiments the glycerol release produced by noradrenaline and this occurred only when the agent was added at a high concentration (500 $\mu$g/ml) which also inhibited theophylline ($10^{-3}$ M) induced lipolysis. These data indicate that in human omental adipose tissue catecholamine-induced lipolysis is mediated by beta adrenergic receptors. They also suggest that the antilipolytic action of high phentolamine concentrations is localized between the formation of cyclic adenosine 3',5' monophosphate (3',5' AMP) and the induction of triglyceride lipase activity.

In all experiments glycerol release produced by noradrenaline was further increased by addition of small concentrations (0.05–100 $\mu$g/ml) of phentolamine to the incubation medium. The stimulation of noradrenaline induced lipolysis was negatively correlated with the effect of noradrenaline alone on basal lipolysis. These small concentrations of phentolamine did not significantly influence the glycerol release produced by theophylline or isopropylnoradrenaline. The maximal lipolytic responses to noradrenaline plus phentolamine were of similar magnitude to those produced either by theophylline or isopropylnoradrenaline alone. These results were interpreted as indicating that in human omental tissue there exist alpha-adrenergic receptors which counteract the lipolytic effect of catecholamines by reducing the formation of 3',5' AMP.

The receptors that mediate th[illegible] fects in various tissues can be [illegible] into alpha and beta types (1). R[illegible] indicate that catecholam[illegible] rat adipose tissue is med[illegible] receptors since it has b[illegible] noradrenaline which stimulates b[illegible] ceptors almost selectively [illegible] a more [illegible] lator of lipolysis than is adrenal[illegible] adrenaline (3, 8, 13). Furthermore [illegible] reported that beta adrenergic blo[illegible] competitively antagonise the effects of catecholamines on lipolysis (5, 8, 9, 17, 20).

Catecholamines are potent lipolytic agents also in human adipose tissue in vitro (4, 7, 10, 11, 15, 16, 24). No studies of the effects of adrenergic blocking agents on catecholamine induced lipolysis in isolated human adipose tissue are available. Recently we have reported that there is in human omental tissue a greater variability between specimens from different subjects in the lipolytic response to noradrenaline than to theophylline, both agents being used at concentrations which induce maximal glycerol release (24). The lipolytic effect of noradrenaline was in some experiments of the same potency as that of theophylline, whereas in other adipose tissues the lipolytic response to noradrenaline was significantly less.

The present studies on the influence of isopropylnoradrenaline and adrenergic blocking agents were undertaken in an attempt to explain what factors may be responsible for such variable in vitro responses of human omental adipose tissue to the lipolytic action of noradrenaline.

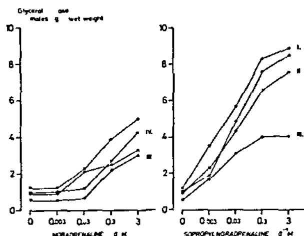



*Fig 1* Comparison between the effects of noradrenaline and isopropylnoradrenaline on the glycerol release from omental tissue in vitro Omental adipose tissue (approx 200 mg) divided into sections of about 50 mg each was incubated for two hours in 3 ml of Krebs Henseleit bicarbonate buffer (pH 7 4) containing 3 % (w/v) of bovine serum albumin and 1 mg/ml of glucose

## MATERIAL AND METHODS

Omental tissue was obtained from patients undergoing cholecystectomy The patients had no clinical signs of diabetes mellitus or other metabolic disorders and had been fasted overnight prior to surgery General anesthesia was induced by ultra short acting barbiturate nitrous oxide and halothane Adipose tissu was removed the start of operation and transferred into albumin buffer solution After preincubation the tissues re placed in media consisting of 3 ml of Krebs-Henseleit bicarbonate buffer (pH 7 4) with 3% (w v) of bovine serum albumin (Armour Pharm Co Eastbourne England Lot number M G 2770) and glucose (100 m / 100 ml)

Lipolysis in adipose tissue was determined by the rate of glycerol release (14 22) from sections of adipose tissue The incubation pro edure was the same as that used in earlier experiments (24) the details of which were reported previously ( 3) Duplicate or triplicate incubations were used in all series of experiments. Statistical calculations have been made according to Snedecor (19)

Agents added in vitro were noradrenaline bitartrate (donated by Astra AB Sodertalje Sweden) isoprenaline sulphate (donated by Riker Laboratories, Loughborough, England) phentolamine HCL as the pure substance (donated by Ciba AB Stockholm, Sweden) propranolol (Inderal® ICI) and theophylline obtained commercially

## RESULTS

Fig 1 illustrates four experiments in which adipose tissue of each of four donors was incubated in the presence of either noradrenaline or isopropylnoradrenaline added at four concentrations (0 003 0 03 0 3 and 3 × 10 M) In all experiments equimolar concentrations of isopropylnoradrenaline produced higher glycerol release than did noradrenaline The differences were significant for each concentration ($p \leq 0.02$) One hundred times greater molar concentration of noradrenaline was required to produce the same lipolytic response as isopropylnoradrenaline Thus there was no significant difference ($p > 0.1$) between the glycerol release produced by 0 003 × 10 M of isopropylnoradrenaline and that produced by 0 3 × 10 M noradrenaline

Fig 2 presents the results from experiments in which we examined the effect of the beta adrenergic blocking agent propranolol on lipolysis induced by 3 × 10 M noradrenaline Glycerol release was inhibited significantly ($p < 0.01$) by propranolol at a concentration of 0 1 µg per ml of the incubation medium and the lipolysis was completely suppressed by 1 µg of propranolol Propranolol (1 µg/ml medium) had no influence on the basal glycerol release or on the lipolysis induced by theophylline (10 M) (Table I)

The alpha adrenergic blocking agent phentolamine produced in all experiments an increase in the noradrenaline induced lipolysis (Fig 3) In some experiments this stimulatory effect of phentolamine was most pronounced when added at concentrations lower than 5 µg whereas in other experiments the maximal glycerol release was produced by high concentrations (5–100 µg/ml) Increase in the phentolamine concentration to 500

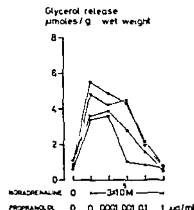



*Fig 2* Effect of different concentrations of propranolol on the noradrenaline (3 × 10⁻⁵ M) induced lipolysis in human omental tissue Incubation conditions are given in Fig 1

Table I *Effect of propranolol on basal and theophylline induced lipolysis*

Omental tissue sections were incubated in 3 albumin medium containing 1 mg ml of glucose with or without theophylline present. Significance is calculated from the paired differences between these media and the same media conta ning propranolol

| Addition to med um | No of experiments | µmol glycerol g wet weight[b] | _ ± S.E.M. |
|---|---|---|---|
| 0 | 8 | 1 07±0 32 | 0 05±0 10 |
| Propranolol | | 1 02±0 49 | |
| Theophylline | 4 | 8 10±0 4 | 0 54±0 0 |
| Theophylline + propranolol | | 7 56±0 36 | |

Propranolol 1 µg ml and theophylline 10 ³ M
[b] Mean ± standard error

µg/ml resulted in decreased glycerol release and occasionally even complete abolition of the glyc erol response to noradrenaline

Phentolamine had a small stimulatory effect on basal lipolysis when added at a concentration of 50 µg/ml ($p < 0.05$) while dosages of 5 and 500 µg/ml did not influence the lipolysis (Table II)

Glycerol release induced by th ophylline (10⁻ M) was regularly blocked by 500 µg of phentol amine whereas a lower concentration of phentol amine had an insignificant effect (Fig 4)

These findings indicate that alpha as well as beta adrenergic receptors are present in human adipose tissue 1) alpha adrenergic receptors with ability to inhibit lipolysis and 2) beta receptors responsible for activation of lipolysis

In order to analyse our results further we have compared the stimulatory effe t of phentolamine (P) on the noradrenaline (NA) induced lipolysis (NA+P/NA) with the effect of noradrenaline alone (NA/Basal) (Fig 5) The values shown in this figure represent the maximal responses ob tained by phentolamine in each experiment given in Fig 3 It appears from the figure that a significant negative relationship exists between these two parameters ($r = 0\ 74$ $p < 0\ 05$) i e the lipolytic effect of noradrenaline is negatively cor related with the potency of the alpha receptor

In five experiments we have compared the maximal glycerol release produced by noradrena line ($3 \times 10^{-}$ M) plus phentolamine with that of theophylline (10 M) (Fig 6) Phentolamine was used in the same concentrations as in previous experiments (Fig 3) There was no significant dif ference between the lipolysis induced by nor adrenaline plus phentolamine and that produced by theophylline In the same experiments (Fig 6) we observed that phentolamine in a concentration of 0 5 µg/ml did not further stimulate the glyc erol release induced by noradrenaline ($3 \times 10$ M) plus theophylline (10 M) Phentolamine in concentrations of 0 5 5 and 50 µg further stimulated the lipolysis induced by noradrenaline added in concentrations of $0\ 03 \times 10^{-}$ M and $3 \times 10^{-}$ M (Fig 7) In these experiments it was shown that phentolamine had no significant in fluence on isopropylnoradrenaline produced lipo- lysis when the latter agent was added at equimolar

Table II *Effect of phentolamine on basal lipolysis in human omental tissue*

Sections of omental tissue were ncubated in dupl cate in 3 albumin med um containing 1 mg ml of glucose and with or without phentolamine present Signif ca e s calculated on the paired differ nces between the two types of med a

| Phentolami e (µg ml) | No of expe ments | µmol glyc ol g wet we ght _ ± S E.M. | P |
|---|---|---|---|
| 5 | 5 | 0 162±0 080 | >0 ∠0 |
| 50 | 6 | 0 19 ±0 073 | <0 05 |
| 500 | 6 | −0 057±0 038 | >0 10 |

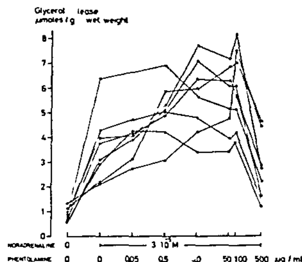



*Fig 3* Effects of different concentrations of the phentolamine on lipolysis induced in human omental tissue with $3\times10^{-6}$ *M* of noradrenaline Incubation procedure is given in Fig. 1

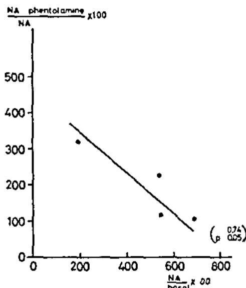



*Fig 5* Relationship between the stimulating effects of noradrenaline on basal lipolysis and of phentolamine on noradrenaline action Maximal lipolysis produced by phentolamine in each series of experiments was used for this calculation Complete experimental data are presented in Fig 3

concentrations Furthermore lipolysis produced by addition of both noradrenaline ($3\times10^{-6}$ *M*) and phentolamine (50 μg/ml) was of equal magnitude to that of isopropylnoradrenaline ($3\times10^{-6}$ *M*)

## DISCUSSION

[Pre]sent data from human omental tissue show [tha]t isopropylnoradrenaline is at least one hundred [ti]mes more potent as a lipolytic agent than is noradrenaline This suggests that beta adrenergic receptors are responsible for the catecholamine induced lipolysis in this tissue Further evidence is the finding that small concentrations of propranolol (1 μg/ml) suppressed completely the noradrenaline induced lipolysis Phentolamine inhibited the noradrenaline effect only when added in a much higher concentration (500 μg/ml) and even then only in some experiments Since phentolamine added at this high concentration inhibited also the lipolysis induced by theophylline one

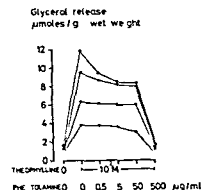



*Fig 4* Effect of phentolamine on glycerol release produced by theophylline ($10^{-3}$ *M*) in human omental tissue

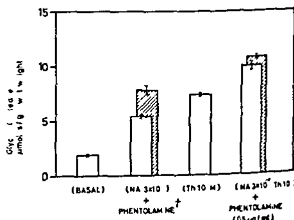


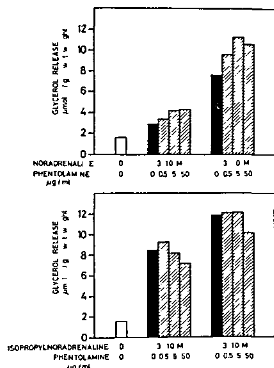

*Fig 6* The effect of phentolamine on lipolysis produced by noradrenaline and noradrenaline plus theophylline ($\bar{X}\pm$S.E.M.) ($n=5$) † = in these experiments the same concentrations of phentolamine were used as in the experiments presented in Fig. 3 Maximal lipolysis produced by phentolamine in each series of experiments has been plotted in this figure

might assume that the agent inhibited lipolysis by interfering with the effect of already formed 3 5 AMP on lipase activity Such an effect has been demonstrated also in isolated rat epididymal fat pad (? 12) In this tissue it has been observed that the lipolytic effect of added dibutyryl 3 5 AMP was also abolished by high concentrations of phentolamine (2)

Our results indicate that alpha adrenergic receptors as well as beta adrenergic receptors exist in human omental adipose tissue It was shown in all experiments that the maximal glycerol release obtained by noradrenaline was further increased by the addition of small concentrations of phentolamine to the incubation medium There is little doubt that this effect of phentolamine is due to an inhibition of the alpha adrenergic receptor since the agent had no influence upon the lipolysis produced by either isopropylnoradrenaline or theophylline Although the stimulatory effect of phentolamine on noradrenaline induced lipolysis has not been demonstrated in rat adipose tissue it is possible that alpha adrenergic receptors might exist in this tissue This is supported by the observation that phentolamine added in concentrations which blocked adrenaline induced lipolysis (18 21) did not antagonise and even occasionally increased the adrenaline effect on the accumulation of 3 5 AMP (6) In other words the specific alpha adrenergic effect of phentolamine in previously reported experiments (18 21) might well have been masked by the nonspecific inhibitory effect of phentolamine on lipolysis

Our experiments demonstrate a significant negative correlation between the maximal stimulatory effect of phentolamine on noradrenaline induced lipolysis ($3 \times 10^{-5}$ *M*) and the pure noradrenaline effect on basal lipolysis This phenomenon could be demonstrated only by the use of different concentrations of phentolamine because the concentration required to produce maximal stimulation varies significantly between omental tissue of different subjects

In the present studies we have mostly used the concentration of noradrenaline ($3 \times 10^{-}$ *M*) which in the majority of cases produces maximal increase in the glycerol release (24) In two experiments it was demonstrated that phentolamine stimulates also the lipolysis produced by concentrations of noradrenaline as low as $0\,03 \times 10^{-5}$ *M* At the present time it is not possible to conclude whether or not the same negative relationship between the noradrenaline effect on basal lipolysis and the stimulatory effect of phentolamine on noradrenaline lipolysis would exist at this more physiologic concentration of noradrenaline




*F g 7* Comparison between the effects of phentolamine on lipolysis induced with noradrenaline and isopropyl noradrenaline in omental tissue Mean of two experiments Incubation procedure is gi en in Fig. 1

At present it is not clear whether the observed differences between the potencies of alpha and beta adrenergic receptors in omental tissue of different patients reflect the situation in vivo or whether they are due to the in vitro conditions per se We are now testing what factors might influence the noradrenaline effect on both types of receptors in vitro

## ACKNOWLEDGEMENTS

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## REFERENCES

1 Ahlquist R. P A study of the adrenotropic receptors. Amer J Physiol 153 586 1948

2 Aulich, A., Stock, K. & Westermann E. Lipolytic effects of cyclic adenosine-3 5 monophosphate and its butyryl derivatives in vitro and their inhibition by α and β adrenolytics. Life Sci 6 9.9 1967
3 Barrett A M The mobilization of free fatty acids in response to isoprenaline in the rat Brit J Pharmacol 25 545 1956
4 Bjorntorp P The fatty acid release and lipolysis of human adipose tissue in vitro Metabolism 14 1318 1964
5 Bray G A Effects of epinephrine corticotropin and thyrotropin on lipolysis and glucose oxidation in rat adipose tissue J Lipid Res. 8 300 1967
6 Butcher R W & Sutherland E. W The effects of the catecholamines, adrenergic blocking agents, prostaglandin E and insulin on cyclic AMP levels in the rat epididymal fat pad in vitro Ann N Y Acad. Sci 139 849 1967
7 Carlson L. A & Hallberg, D Basal lipolysis and effects of norepinephrine and prostaglandin E on lipolysis in human subcutaneous and omental adipose tissue J Lab clin Med 71 368 1968
8 Fain, J N Adrenergic blockade of hormone induced lipolysis in isolated fat cells. Ann N Y Acad Sci 139 879 1967
9 Fassina G Effects on lipomobilization of the β adrenergic blocking drugs, propranolol and INPEA. J Pharm Pharmacol 18 399 1966
10 Galton D J & Bray G A Studies on lipolysis in human adipose cells. J clin Invest 46 621 1967
11 Hamosh M Hamosh P Bar Maor J A & Cohen, H Fatty acid metabolism by human adipose tissues. J clin Invest. 4. 1648 1963
12 Krishna G Hynie S & Brodie B B Theophylline—a tool in the study of mechanism of inhibition lipolysis by adrenergic blocking agents. Pharmacologist 8 195 1966
A M Arnold A McAuliff J P Luduena, F P & Brown, T G Jr Differentiation of receptor systems activated by sympathomimetic amines. Nature .14 597 1967
14 Larsen, J A Elimination of glycerol as a measure of the hepatic blood flow in the cat Acta physiol scand 57 2.4 1963
15 Martinsson, A Release of glycerol and FFA in human adipose tissue in vitro Acta med scand. 18. 787 1967
16 Mosinger B., Kuhn, E & Kujalova, V Action of adipokinetic hormones on human adipose tissue in vitro J Lab clin. Med. 66 380 1965
17 Muhlbachová E., Wenke M., Hynie S & Dolejsova, K. Interactions of some sympathicotropic agents affecting lipid mobilization Arch. int Pharmacodyn. 144 454 1963
18 Scholz, M C. & Page, J H. The effect of adrenergic blocking agents on the release of free fatty acids from rat adipose tissue. J Lipid Res. 1 466 1960
19 Snedecor G W Statistical methods. Iowa State College Press, Iowa 1957
20 Stock, K. & Westermann, E. Competitive and non competitive inhibition of lipolysis by α and β receptor blocking agents, methoxamine derivatives and prostaglandin $E_1$ Life Sci 5 1667 1966
21 Wenke M Muhlbachová, E & Hynie S Effects of some sympathicotropic agents on the lipid metabolism. Arch. int. Pharmacodyn 136 104 196.
22. Wieland O Eine enzymatische Methode zur Bestimmung von Glycerin. Biochem. Z. 329 313 1957
.3 Östman, J A procedure for in vitro studies on fatty acid metabolism by human subcutaneous adipose tissue Acta med scand 177 183 1965
.4 Östman J Efendic, S & Arner P Catecholamines and metabolism of human adipose tissue I Comparison between in vitro effects of noradrenaline, adrenaline and theophylline on lipolysis in omental adipose tissue Acta med scand. 186 .41 1969

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# CATECHOLAMINES AND METABOLISM OF HUMAN ADIPOSE TISSUE

## III Comparison between the Regulation of Lipolysis in Omental and Subcut ne us Adipose Tissue

Suad Efendić

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*Abst act* The effects on lipolysis of *l* noradrenaline *l* isopropylnoradrenaline theophylline and N 2 0-dibuty yl adeno ine 3 5 monophosphate have been studied on sections of human subcutaneous and omental adipose tissue obtained from 17 subjects undergoing abdominal surgery

In both tissues isopropylnoradrenaline an almost pure beta adrenergic stimulator induced higher glycerol re lease than noradrenaline The alpha adrenergic blocking agent phentolamine increased the noradrenaline stimu lated glycerol release in both tissues These data indi cate that catecholamine induced lipolysis is mediated by beta adrenergic receptors and is inh bited by alpha re ceptor

Lipolysis induced by e ther noradrenal ne or isopro pylnoradrenaline was greater in omental than in sub cutaneous adipose tissue On the other hand theophyl line which is supposed to increase the tissue level of cyclic AMP and administration of cyclic AMP itself (the dibutyryl form) induced almost equal lipolysis in both tissues. It is therefore suggested that the lower lipolytic response to catecholamines of subcutaneous adi pose tissue is due to a lower format on of cyclic AMP

In r cent studies w demonstrated for the first tim that in human omental adipose tissue both alpha and beta adrenergic receptors play a rol in th lipolytic activity of catecholamines (7 8) It was shown that noradrenaline stimulated lipo lysis more actively in human omental than sub cutan ous adipose tissue (1 2 4) The pr sent work was undertaken in order to study furth r this difference b tween omental and subcutan ous adipose tissue in their lipolytic response to noradrenalin with sp cial emphasis on the sig nificance of adrenergic receptors for its regula tion

## MATERIAL AND METHODS

Adipose tis ue was obta.ned from 17 patient 11 females and six males with the a e range 25 to 75 year under going abdominal surgery (cholec stectomy and gastr re section because of stomach ulcer) The pat e t were fasting overnight and premed cated before oper o  w th atropin and Petidin® (ACO Stockholm Sweden) An esthesia was induced with short acting barbiturate (Nar kotal® Astra Sweden) and continued with Haloth n ® (ICI England) N O and O in combination with suxo methonium chloride (Celocurin® V trum Sweden) Samples of adipose tissue were obtained 0–50 min after the start of operat on Dur ng this time saline but not glucose was infused intravenou ly The subcutaneous tis sue was taken from the region of incision and the omen tal from the major omentum The ad pose tissue was immediately placed into Krebs Henseleit bicarbonate (KHB) buffer (pH 7 4) contain ng 1 (W/V) of bo ine serum album n at 37 C and brought to the laboratory The tissues were divided into sections of approximately 150–200 mg and pre ncubated for 60 m n n the same med um After preincubation the sect ons were placed on filter paper blotted w th saline cleaned from visible connective tissue and blood vessels div ded into pieces weighing about 50 mg each and transferred into plastic vials (Packard) About 200 mg of adipose t ssue was incubated in each vessel The basal incubation med um was 3 ml of KHB buffer (pH 7 4) contain ng 3 bov ne albumin (Fract on V Armour Pharmaceut cal Di ision lot number MG 2770) prov ding approximately 0 35 μmole of FFA per ml medium and 1 mg/ml of glu co e All ncubat ons were run in d plicate After two hours of incubat on al quots of med um were removed for glycerol determination accord ng to Wieland (6) as modif ed by Larsen (3) Statist cal calculat ons ha e been made according to Snedecor (5)

Agents added in vitro were noradrenal ne bitartrate (from Astra AB Sodertalje Sweden) *l* isopropylnoradre nal ne-*d* bitartrate dihydrate (from Sterl ng Winthrop Re search Inst tute) phentolamine HCl as a pu e substa

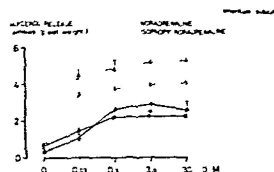



*Fig 1* Comparison of lipolytic effects of different concentrations of noradrenaline and isopropylnoradrenaline in omental and subcutaneous adipose tissue. Adipose tissue (approx. 200 mg) was divided into sections of about 0 mg and incubated for two hours in 3 ml of Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 3% (W V) of bovine serum albumin. Each point represents the mean and standard error of five experiments, each run in duplicate.

(from Ciba AB Stockholm, Sweden), N⁶2 0-dibutyryl-adenosine 3,5-monophosphate (Biochemica Boehringer Mannheim, West Germany) and theophylline obtained commercially.

## RESULTS

Fig. 1 presents the results of five experiments in which were compared the lipolytic effects of equimolar concentrations of noradrenaline and isopropylnoradrenaline in omental and subcutaneous adipose tissue. In both tissues isopropylnoradrenaline at each concentration induced higher glycerol release than noradrenaline. Isopropylnoradrenaline added in a concentration as low as $0.03 \times 10^{-6}$ M induced almost maximal stimulation of lipolysis in both types of tissues and concentrations up to one thousand times higher produced only slight further increases. The noradrenaline concentration ($0.3 \times 10^{-6}$ M) required to induce maximal lipolysis was also the same for omental and subcutaneous adipose tissue. Both agents, except for the lowest examined concentration of noradrenaline ($0.03 \times 10^{-6}$ M) induced higher glycerol release from omental than from subcutaneous adipose tissue.

Table I summarizes the results of 17 experiments in which we determined basal lipolysis and maximal lipolysis induced with noradrenaline or isopropylnoradrenaline in human omental and subcutaneous adipose tissue. Basal glycerol release was higher from subcutaneous adipose tissue in sixteen of these experiments. The mean difference between omental and subcutaneous tissue was highly significant ($p<0.001$). On the contrary maximally stimulated lipolysis was higher from omental tissue both for noradrenaline ($p<0.005$) and for isopropylnoradrenaline ($p<0.001$). On the average noradrenaline produced 47% and isopropylnoradrenaline 43% higher glycerol release from omental adipose tissue. In Fig. 2 is plotted the basal glycerol release against the glycerol release stimulated with either noradrenaline or isopropylnoradrenaline. In omental tissue these parameters are significantly correlated ($p<0.02$ for noradrenaline and $p<0.001$ for isopropylnoradrenaline) whereas in subcutaneous tissue significant correlation was observed only between basal and noradrenaline-stimulated lipolysis ($p<0.001$). Positive correlation existed between subcutaneous and omental tissue in their lipolytic responses to noradrenaline ($r=0.54$, $p<0.05$) as well as to isopropylnoradrenaline ($r=0.52$, $p<$

Table I. *Comparison between maximal lipolytic effects of noradrenaline (NA) and isopropylnoradrenaline (ISPNA) in human omental and subcutaneous adipose tissue*

(M ± S.E., N = 17)

| Origin of adipose tissue | Glycerol release[a] Basal | NA $3 \times 10^{-6}$ M | NA $3 \times 10^{-6}$ M – basal | ISPNA $3 \times 10^{-6}$ M | ISPNA $3 \times 10^{-6}$ M – basal |
|---|---|---|---|---|---|
| Omental | 0.47 ± 0.05 | 3.07 ± 0.25 | 2.8 ± 0.23 | 5.20 ± 0.34 | 4.7 ± 0.31 |
| Subcutaneous | 0.8 ± 0.04 | 2.3 ± 0.1 | 1.45 ± 0.14 | 3.6 ± 0.16 | 2.87 ± 0.15 |
| Mean diff. ± S.E.M. | −0.27 ± 0.07 | 0.84 ± 0.2 | 1.12 ± 0.20 | 1.53 ± 0.30 | 1.88 ± 0.4 |
| $p$ | 0.005 | 0.005 | 0.001 | 0.001 | 0.001 |

[a] Values are expressed as μmoles of glycerol g wet weight⁻¹. Incubation conditions as given in Fig. 1.

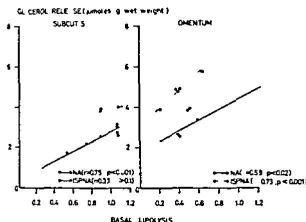



*Fig 2* Relationship between basal lipolysis and lipolysis stimulated with noradrenaline (NA) and isopropylnoradrenaline (ISPNA) in subcutaneous and omental adipose tissue Regression equations
NA ($3 \times 10^{-6}$ M)
subcutis $Y = .43\ X + 0\ 34$
omentum $Y = 2.64\ X + 1\ 78$
ISPNA ($3 \times 10^{-6}$ M)
subcutis $Y = 0\ 98\ X + 2\ 90$
omentum $Y = 4\ 41\ X + 3\ 05$
Incubation conditions are given in Fig 1

05) (Fig 3) The aforementioned slopes (Fig 3) were not significantly different ($p > 0\ 1$) No correlation was found between the basal glycerol releases.

In order to study the mechanisms behind the less pronounced lipolytic response to catecholamines in subcutaneous adipose tissue we compared in twelve experiments the lipolytic effects of noradrenaline ($3 \times 10^{-}$ M) and isopropylnoradrenaline ($3 \times 10^{-5}$ M) with those of theophyllin ($10^{-}$ M) and dibutyryl cAMP ($10^{-3}$ M) on subcutaneous and omental adipose tissue since these

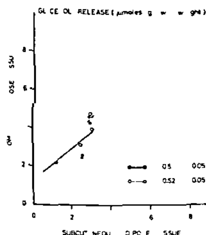



*Fig 3* Relationship between noradrenaline (NA) and isopropylnoradrenaline (ISPNA) effects on glycerol release (µmoles g weight . h) from human subcutaneous (X) and omental (Y) adipose tissue Regression equations
NA ($3 \quad 10 \quad M$) (●— —●)
$Y = 0\ 80\ X + 1\ 9$
ISPNA (○— —○)
$Y = 1\ 13\ X + 1\ 04$

latter agents act by different mechanisms on lipolysis In separate experiments we have established that the same concentrations of isopropylnoradrenalin theophyllin and dibutyryl cAMP produced almost the same degree of stimulation of lipolysis in human omental adipose tissue Our results (Table II) show that in these experiments the lipolytic effects of catecholamines were significantly higher in omental tissue ($p < 0\ 02$) ($p < 0\ 001$) whereas lipolysis induced with theophylline and dibutyryl cAMP were of the same magnitude in both tissues When the true lipolytic effects (stimulated lipolysis—basal lipolysis) were calculated the dibutyryl cAMP induced lipolysis was slightly higher in omental adipose tissue ($p < 0\ 05$) In both tissues dibutyryl cAMP and theophylline induced lipolysis were highly correlated ($p < 0\ 001$) (Fig 4) the correlation coefficient being almost one for both tissues In omental adipose tissue isopropylnoradrenaline induced lipolysis was significantly correlated with theophylline and dibutyryl cAMP induced lipolysis ($p < 0\ 005$) (Fig 5) On the other hand these parameters were not correlated in subcutaneous adipose tissue

In ten experiments the influence of the alpha adrenergic blocking agent phentolamine (0 5 and 50 µg ml) on noradrenaline ($3 \times 10^{-}$ M) induced lipolysis was studied (Table III) These concentrations of phentolamine were used since it was previously shown (8) that in most omental tissues maximal stimulation of noradrenaline effect is obtained with either low (0 5 µg) or high (50 µg) phentolamine concentration In Table III are given data where higher glycerol release was obtained independently of the concentration of phentolamine used Our results show that ph

Table II *Comparison between lipolytic effects of nor[illegible] A) isopropylnoradrenaline (ISPNA) theophylline (Th) and dibutyryl cAMP (dAMP) in human omental and subcutaneous adipose tissue*

(*M*±S.E. *N*=12)

| Origin of adipose tissue | Glycerol release[a] Basal | NA 3 10[illegible] *M* | NA 3 10[illegible] *M* basal | ISPNA 3 10[illegible] *M* | ISPNA 3×10[illegible] *M* – basal | Th 10[illegible] *M* | Th 10[illegible] *M* – basal | dAMP 10[illegible] *M* | dAMP 10[illegible] *M* – basal |
|---|---|---|---|---|---|---|---|---|---|
| Omental | 0 54±0 07 | 3 1[illegible]±0 32 | 2 59±0 27 | 5 19±0 48 | 4 65±0 43 | 4 84±0 60 | 4 31±0 55 | 5 14±0 69 | 4 60±0 64 |
| Subcutaneous | 0 81±0 07 | 2 22±0 [illegible] | 1 40±0 17 | 3 56±0 [illegible]0 | 2 75±0 18 | 4 44±0 37 | 3 63±0 42 | 4 23±0 44 | 3 42±0 49 |
| Mean diff ± S.E.M | −0 [illegible]8±0 09 | 0 91±0 30 | 1 19±0 [illegible]4 | 1 63±0 40 | 1 90±0 38 | 0 40±0 46 | 0 68±0 42 | 0 91±0 53 | 1 19±0 50 |
| *p* | 0 0 | 0 02 | 0 001 | 0 005 | <0 001 | >0 05 | >0 05 | >0 05 | <0 05 |

[a] Values are expressed as μmoles/g wet weight/2 h
Incubation conditions are given in Fig. 1

tolamine significantly ($p<0.01$) stimulated noradrenaline induced lipolysis in both tissues. The effect of noradrenaline was increased by 53% in omental and by 44% in subcutaneous tissue this difference being insignificant In omental tissue lipolysis induced with noradrenaline in the presence of phentolamine was not different ($p>0.05$) from the maximal lipolysis induced with isopropylnoradrenaline However in subcutaneous adipose tissue isopropylnoradrenaline induced lipolysis was in all but one experiment higher than that achieved with noradrenaline plus phentolamine the statistical significance being $p<0.02$.

## DISCUSSION

We have recently shown that in human omental adipose tissue there exist alpha as well as beta adrenergic receptors which influence lipolysis in opposite directions (8) Stimulation of alpha adrenergic receptors inhibited whereas beta adrenergic receptors activated lipolysis The present results confirm these data and furthermore indicate that the same relationship between lipolysis and the stimulation of adrenergic receptors exists also in subcutaneous adipose tissue In favor of such a statement is the finding that isopropylnoradrenalin was a more potent agent than noradrenaline at all examined concentrations in both tissues Isopropylnoradrenaline in a concentration as low as $0.03 \times 10^{-6}$ M induced maximal stimulation of lipolysis while a ten times higher concentration of noradrenaline was required for maximal effect ($0.3 \times 10^{-5}$ *M*)

Further support for the above statement was the finding that the alpha adrenergic blocking agent phentolamine (0.5 or 50 μg/ml) significantly stimulated lipolysis in both tissues Using a wide range of phentolamine concentrations we previously found that in omental adipose tissue maximal stimulation of noradrenaline induced lipolysis occurred in the presence of either 0.5 or 50 μg/ml of phentolamine (8) In the present experiments it was observed in this tissue that lipolysis induced with noradrenaline and phentolamine (0.5 and 50 μg/ml) was of the same magnitude as maximal lipolysis induced with isopropylnoradrenaline This was not the case in subcutaneous adipose tissue where isopropylnoradrenaline produced significantly higher glycerol release than noradrenaline plus phentolamine

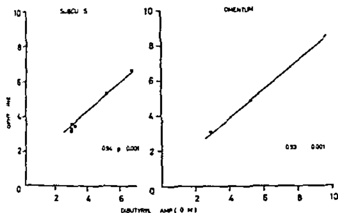


*Fig 4* Relationship between dibutyryl cAMP (*X*) and theophylline (*Y*) induced glycerol releases in human subcutaneous and omental adipose tissue Regression equations
subcutis $Y = 0\ 80\ X - 1\ 07$
omentum $Y = 0\ 81\ X - 0\ 66$
Incubation conditions are given in Fig. 1

<0 0?) This is probably due to the fact that with the concentrations of phentolamine used we were not able to obtain maximal stimulation of noradrenaline induced lipolysis in subcutaneous tissues with its generally lower lipolytic response to the addition of catecholamines.

Our data show that in omental adipose tissue a lower maximal lipolytic response to noradrenaline than to isopropylnoradrenaline is due to the inhibition of lipolysis by alpha adrenergic receptors. Most likely this is also true for subcutaneous adipose tissue. In addition our data suggest that the ratio between the relative potencies of alpha and beta adrenergic receptors is almost equal in both tissues As seen in Fig. 3 the regression lines denoting the ratio of glycerol release between subcutaneous and omental adipose tissue induced by isopropylnoradrenaline and noradrenaline were not significantly different Since the difference between the two drugs in this respect lies in the alpha adrenergic response induced by noradrenaline the observation indicates that a decrease in beta adrenergic stimulation in subcutaneous adipose tissue is accompanied by a proportional decrease in alpha adrenergic response

The present observations strongly suggest that a rate limiting step in catecholamine induced lipolysis in subcutaneous adipose tissue is the activation of adrenergic receptors, and consequently decreased formation of cyclic AMP This statement is supported by experiments in which the lipolytic response to addition of theophylline and dibutyryl cAMP was studied These agents stimulated the rate of glycerol release to almost the same extent in tissue from the two localisations In addition theophylline and dibutyryl cAMP induced glycerol releases were highly correlated in both tissues ($r = 0\ 94$ and $0\ 93$) whereas the isopropylnoradrenaline induced lipolysis was correlated with the dibutyryl cAMP and theophylline effects only in omental adipose tissue

In omental adipose tissue the basal glycerol re

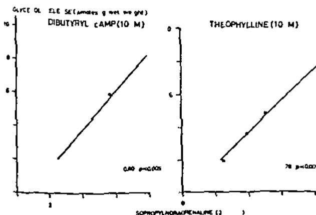



*Fig 5* Relationship between isopropylnoradrenaline (*X*) induced glycerol release and the glycerol release induced with dibutyryl cAMP (*Y*) and theophylline (*Y*) in omental adipose tissue Regression equations

(*a*) isopropylnoradrenaline–dibutyryl cAMP
$Y = 1\ 17\ X - 0\ 9$

(*b*) isopropylnoradrenaline–theophylline
$Y = 0\ 98\ X - 0\ 26$

Table III *Comparison between noradrenaline (NA) [illegible] plus phentolamine (NA + Ph) and isopropylnoradrenaline (ISPNA) induced lipolysis in human omental and subcutaneous adipose tissue*

| No of experiment | Glycerol release[a] Omental a t NA | NA + Ph | ISPNA | NA + Ph / NA 100 | ISPNA / NA 100 | Subcutaneous a t NA | NA + Ph | ISPNA | NA + Ph / NA 100 | ISPNA / NA × 100 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 61 | [illegible] 67 | 1 88 | 10[illegible] | 149 | 2 58 | 3 27 | 3 92 | 127 | 152 |
| 2 | 2 69 | 4 8[illegible] | 5 76 | 179 | 214 | 1 [illegible]0 | 3 29 | 3 47 | 274 | [illegible]89 |
| 3 | 2 54 | 4 00 | 4 89 | 157 | 193 | 2 61 | 3 44 | 4 00 | 132 | 153 |
| 4 | 2 44 | 4 46 | 3 9[illegible] | 181 | 161 | 1 86 | [illegible] 57 | 3 15 | 138 | 169 |
| 5 | 3 10 | 7 40 | 7 49 | 224 | 227 | 2 19 | 4 03 | 4 30 | 184 | 196 |
| 6 | 3 65 | 4 82 | 5 76 | 132 | 158 | 1 99 | 2 71 | 3 04 | 136 | 151 |
| 7 | 3 38 | 3 76 | 5 15 | 111 | 158 | 3 14 | 3 15 | 3 77 | 100 | 1 0 |
| 8 | 1 60 | 3 10 | 2 45 | 194 | 153 | 2 74 | 3 22 | 3 5[illegible] | 118 | 1 8 |
| 9 | 4 6[illegible] | 4 58 | 5 07 | 99 | 110 | [illegible]04 | 2 69 | 2 76 | 88 | 91 |
| 10 | 4 47 | 6 62 | 6 75 | 148 | 151 | 75 | 3 95 | 3 47 | 144 | 1 6 |
| Mean ± s e m | 3 13±0 30 | 4 6 ±0 46 | 5 13±0 46 | 153± 13 | 167± 11 | [illegible] 41±0 19 | 3 23±0 15 | 3 54±0 15 | 144± 17 | 158± 17 |
| Mean diff ± s e m and *p* | 1 49±0 39 0 005 | | 0 50±0 21 >0 05 | 14 50±9 38 0 05 | | 0 82±0 23 0 01 | | 0 31±0 11 0 02 | 13 50±4 40 <0 02 | |
| | | | | | | 7 7±14 >0 1 | | | | |
| | | | | | | | | 9 7±11 >0 1 | | |

[a] Values are expressed as μmoles g wet weight [illegible] h NA conc 3 × 10 [illegible] M ISPNA conc 3 × 10 [illegible] M Ph conc 0 5 and 50 μg/ml
Incubation conditions are given in Fig 1

lysis was correlated with glycerol releases induced by either noradrenaline or isopropylnoradrenaline. This would indicate that the basal and the stimulated lipolysis are controlled by the same mechanism, presumably the formation of cyclic AMP. In accordance with data previously reported by Carlson and Hallberg (1) we observed significantly higher basal glycerol release from subcutaneous adipose tissue. One explanation for this would be that the rate of cyclic AMP formation in subcutaneous adipose tissue is higher under basal conditions. Recently Micheli et al. (4) in a material of 39 patients observed no difference in basal lipolysis between omental and subcutaneous adipose tissue. These controversial results are probably due to differences in the clinical material in the two studies since the same incubation procedures, premedication and anesthesia were used. In subcutaneous tissue we have found significant positive correlation between basal lipolysis and lipolysis stimulated with noradrenaline. This indicates that lipolysis here is also controlled by cyclic AMP formation. On the other hand there was no correlation in this tissue between basal lipolysis and lipolysis induced with isopropylnoradrenaline. The explanation for this would be that the basal lipolysis in some specimens of subcutaneous adipose tissue is so high that addition of such a potent lipolytic agent as isopropylnoradrenaline produces relatively less increase in lipolysis because of previous saturation of lipase activity.

## ACKNOWLEDGEMENTS

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## REFERENCES

1. Carlson, L. A. & Hallberg, D.: Basal lipolysis and effects of norepinephrine and prostaglandin $E_1$ on lipolysis in human subcutaneous and omental adipose tissue. J. Lab. clin. Med. 71: 368, 1968.
2. Carlson, L. A., Hallberg, D. & Micheli, H.: Quantitative studies on the lipolytic response of human subcutaneous and omental adipose tissue to noradrenaline and theophylline. Acta med. scand. 185: 465, 1969.
3. Larsen, J. A.: Elimination of glycerol as a measure of the hepatic blood flow in the cat. Acta physiol. scand. 57: 224, 1963.
4. Micheli, H., Carlson, L. A. & Hallberg, D.: Comparison of lipolysis in human subcutaneous and omental adipose tissue with regard to effects of noradrenaline, theophylline, prostaglandin $E_1$ and age. Acta chir. scand. In press.
5. Snedecor, G. W.: Statistical methods. Iowa State College Press, Iowa 1957.
6. Wieland, O.: Eine enzymatische Methode zur Bestimmung von Glycerin. Biochem. Z. 329: 313, 1957.
7. Östman, J., Efendic, S. & Arner, P.: Catecholamines and metabolism of human adipose tissue. I. Comparison between in vitro effects of noradrenaline, adrenaline and theophylline on lipolysis in omental adipose tissue. Acta med. scand. 186: 241, 1969.
8. Östman, J. & Efendic, S.: Catecholamines and metabolism of human adipose tissue. II. Effect of isopropylnoradrenaline and adrenergic blocking agents on lipolysis in human omental adipose tissue in vitro. Acta med. scand. 187: 471, 1970.

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# CATECHOLAMINES AND METABOLISM OF HUMAN ADIPOSE TISSUE

## IV *Influence of Glucose on Catecholamine Induced Lipolysis in Human Omental Adipose Tissue in vitro*

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*Abstract* The influence of glucose on the in vitro lipolysis of human omental adipose tissue has been studied Omental tissue obtained at surgery during general anesthesia was incubated in Krebs-Henseleit bicarbonate buffer containing 3 bovine albumin with and without added glucose (2 mg/ml) Glycerol release into the medium was considered as an index of lipolysis. Glucose enhanced the basal lipolysis and potentiated significantly the adipokinetic effects of noradrenaline isopropylnoradrenaline and N 2 0-dibutyryl cAMP (dibutyryl cAMP) The slope of glycerol release obtained for dibutyryl cAMP in glucose free versus glucose-containing medium was significantly different from those obtained for noradrenaline and isopropylnoradrenaline This difference might be explained if it is admitted that glucose stimulates lipolysis by acting on two different sites (*a*) close to the triglyceride-splitting enzyme system and (*b*) close to the beta adrenergic receptor

In a second series of experiments the effect of glucose on the level of FFA was studied in tissue and in medium with noradrenaline present. No changes in the net release of FFA were observed whereas a slight increase in the tissue level of FFA was demonstrated. These findings in human tissue are not in accordance with those previously described in rat epididymal fat pad and do not justify the assumption that a decrease in the tissue level of FFA is the trigger mechanism for the lipolysis promoting effect of glucose

A marked decrease in the glycerol release was noticed when 2-deoxy-*d* glucose was added to medium containing noradrenaline isopropylnoradrenaline and dibutyryl cAMP The data are consistent with the view that glucose metabolism and the activity of triglyceride lipase are closely related

A number of studies have shown that the addition of glucose to the medium during in vitro incubation of rat adipose tissue decreases release of free fatty acids presumably due to an increased rate of re-esterification of FFA (2 3 6 7 12 13 19) In addition it is well established that glucose stimulates basal as well as catecholamine induced glycerol release from rat epididymal fat pad (3 7 8 9 14) Since glycerol production in rat adipose tissue reflects lipolysis (20) this finding has been interpreted as demonstrating an increase in lipolysis Jungas and Ball (14) suggested that by furnishing glycerophosphate for the re esterification process glucose lowers the level of FFA within tissue to such an extent that lipolysis is favored Recently Chlouverakis (9) reported that among different glucose metabolites including glycerophosphate only fructose 1–6 diphosphate induced a significant stimulation of lipolysis This observation speaks in favor of a direct effect of glucose or its metabolites on lipolysis

This paper presents data showing that glucose increases lipolysis both under basal conditions and when lipolysis in human omental adipose tissue in vitro is stimulated by different agents These agents were noradrenaline isopropylnoradrenaline and $N^6$ 2 0-dibutyryl cAMP (dibutyryl cAMP) The main conclusion from these studies is that there is a dual localization of the lipolysis-promoting action of glucose (*a*) close to the triglyceride splitting enzyme system and (*b*) close to the beta adrenergic receptor

## MATERIAL AND METHODS

Omental tissue was obtained from patients undergoing abdominal surgery mostly cholecystectomy None of the patients was acutely ill and none had clinical evidence of endocrine disease including diabetes. The taken in the morning after fasting o

Table I *Influence of glucose on basal and noradrenaline induced glycerol release (Mean ± S.E. n = 20)*

Omental adipose tissue (approx. 200 mg) divided into sections of about 50 mg each was incubated for two hours in 3 ml of Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 3 (w/v) of bovine serum albumin

| Glucose concentration | Glycerol release[a] Basal | NA $3\times10^{-6}$ M | NA $3\times10^{-6}$ M − basal |
|---|---|---|---|
| 0 | 0.48 ± 0.04 | 2.09 ± 0.16 | 1.61 ± 0.17 |
| 2 mg/ml | 0.75 ± 0.04 | 3.35 ± 0.11 | 2.59 ± 0.12 |
| Mean diff ± S.E.M.[b] | 0.27 ± 0.03 | 1.26 ± 0.09 | 0.98 ± 0.11 |
| $p$ | < 0.001 | < 0.001 | < 0.001 |

[a] µmoles/g wet weight/2 h

[b] Glucose-containing − glucose free medium

anesthesia was induced by ultra short acting barbiturate nitrous oxide and halothane. The procedure for handling of adipose tissue prior to and during incubation has been described previously (2, 23). In all experiments parallel incubations, without and with added glucose (2 mg/ml incubation medium) were performed in duplicate. Aliquots of the medium were removed for determination of glycerol according to Wieland (21) as modified by Larsen (15). In some experiments the medium and tissue FFA were determined according to the Dole procedure (10) as modified by Trout et al. (18). Statistical calculations have been made according to Snedecor (17)

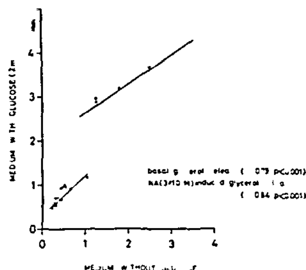


*Fig 1* Comparison between the basal and noradrenaline ($3\times10^{-6}$ M) stimulated glycerol release in absence and presence of glucose (2 mg/ml). Values are expressed as µmoles glycerol/g wet weight/2 h incubation. Regression equations: basal conditions $Y = 0.88X + 0.33$; NA ($3\times10^{-6}$ M) $Y = 0.66X + 1.97$. Incubation conditions are given in Table I

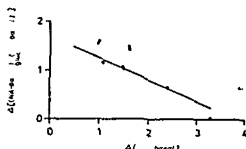


*Fig 2* Relationship between the true effect of noradrenaline ($3\times10^{-6}$ M) on glycerol release in glucose *free medium and the true effect of glucose on noradrenaline induced lipolysis*. Values are expressed as µmoles glycerol/g wet weight/2 h incubation. Regression equation $Y = -0.44X + 1.69$. Δ(NA-basal) = noradrenaline induced glycerol release—basal glycerol release in glucose free medium. Δ(NA-basal)[illegible] = noradrenaline induced glycerol release—basal glycerol release in glucose-enriched medium (2 mg glucose/ml). Incubation conditions are given in Table I

Agents added in vitro were *l*-noradrenaline bitartrate (Astra AB, Södertälje, Sweden), *d*, *l*-isoprenaline sulfate (*Riker Laboratories, Loughborough, England*) and N⁶ 2′O-dibutyryl cAMP (Biochemica Boehringer, Mannheim, West Germany)

## RESULTS

Table I presents the results from 20 experiments showing the influence of glucose (2 mg/ml) on basal lipolysis and on lipolysis induced with noradrenaline in a concentration known to produce maximal glycerol release ($3\times10^{-6}$ M). Under both conditions the addition of glucose increased the rate of glycerol release by about 60%. Furthermore the "true" effect of noradrenaline i.e. *glycerol release in the presence of noradrenaline* minus basal glycerol release was significantly higher ($p < 0.001$) in the presence of glucose. Fig. 1 depicts the results in individual experiments. The correlation coefficients for glycerol release between glucose poor and glucose-enriched medium were for basal glycerol release ($r = 0.79$, $p < 0.001$) and for noradrenaline produced glycerol release ($r = 0.84$, $p < 0.001$). The glucose noradrenaline interrelationship was further analyzed by calculating the slope and correlation coefficient *between the true effect of noradrenaline* in glucose free medium versus the potentiating effect of glucose on the noradrenaline response. It is shown in Fig. 2 that these parameters *are negatively* correlated ($r = -0.67$, $p < 0.005$). This means

Table II *Influence of glucose on dibutyryl cAMP (dAMP) induced lipolysis (Mean ± S E )*

Incubat on conditions are given in Table I

| Clucose concentration | Glycerol release[a] dAMP $10^{-4}$ M | dAMP $3\times10^{-4}$ M | dAMP $10^{-3}$ M | dAMP $10^{-3}$ M – basal |
|---|---|---|---|---|
| 0 | 1 01±0 11 | 1 65±0 17 | 3 60±0 46 | 3 10±0 53 |
| 2 mg ml | 1 34±0 05 | 2 13±0 19 | 4 57±0 57 | 3 67±0 60 |
| Mean diff ± S E M [b] | 0 33±0 15 ($n=7$) | 0 48±0 21 ($n=7$) | 0 96±0 19 ($n=9$) | 0 57±0 16 ($n=9$) |
| $p$ | >0 05 | >0 05 | <0 005 | <0 01 |

[a] μmoles g wet weight/2 h
[b] *Glu ose-containing – glucose free medium*

that the glucos effect on noradrenaline induced lipolysis tends to be more pronounced in tissues showing poor response to noradr naline

In order to localize the site of effect of glucose in the lipolytic machinery studi s were performed on the influence of glucose on lipolysis induced with N[6] 2 0-dibutyryl cyclic AMP (dibutyryl cAMP) an agent which apparently stimulates li polysis by direct activation of the lipolytic en zym system (1) Addition of glucose (2 mg/ml) stimulat d significantly ($p<0\ 005$) lipolysis in duced with dibutyryl cAMP added in a concen tration of $10^{-3}$ M but had no effect when dibu tyryl cAMP was present at lower concentrations ($10^{-4}$ and $3\times10^{-4}$ M) The true effect of dibu tyryl cAMP ($10^{-3}$ M) was also significantly higher ($p<0\ 01$) in glucose milieu Fig 3 demon strates that a linear relationship ($r=0\ 97$ $p<0\ 001$) existed between dibutyryl cAMP induced glycerol release in the pres nce of glucose as com pared to response in m dia deprived of glucose Comparison of the aforementioned slop s indi cates that glucose affected lipolysis differently wh n the tissue was stimulated with noradrenaline than when stimulated with dibutyryl cAMP The difference betw en slopes (Figs 1 and 3) is sig nificant ($p<0\ 001$)

In some experiments we have incubated tissue during different p riods of time in order to find

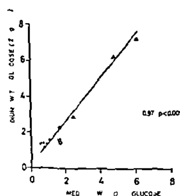
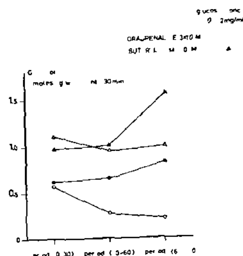



*Fig 3 Relationship between glycerol release induced with* different concentrations of N[6] 2 0-dibutyryl cAMP (dAMP) in med a without and with added glucose (2 mg/ml) Values are expressed as μmoles glycerol/g wet *weight/2 h incubation. Regress on equation Y = 1 19X* +0 0 $10^{-4}$ M dAMP (N = 7) o $3\times10^{-4}$ M dAMP (N = 7) Δ $10^{-3}$ M dAMP (N = 9) Incubation conditions are given in Table I




F g 4 Effect of glucose on the lipolytic responses to noradrenaline and dibutyryl cAMP in omental tissue during arious periods of incubation ($n=3$)

Table III *Influence of glucose on isopropylnoradrenaline (ISPNA) induced lipolysis* (Mean ± S.E.)
Incubation conditions are given in Table I

| Glucose concentration | Glycerol release[a] ISPNA $3\times10^{-7}$ M | ISPNA $3\times10^{-5}$ M | ISPNA $3\times10^{-7}$ M – basal | ISPNA $3\times10^{-5}$ M – basal |
|---|---|---|---|---|
| 0 | 2.30±0.19 | 3.06±0.34 | 1.71±0.17 | 2.44±0.30 |
| 2 mg/ml | 3.37±0.23 | 4.51±0.16 | 2.41±0.22 | 3.46±0.27 |
| Mean diff ± S.E.M.[b] | 1.07±0.15 ($n=11$) | 1.44±0.32 ($n=8$) | 0.70±0.13 ($n=11$) | 1.02±0.31 ($n=8$) |
| $p$ | <0.001 | <0.005 | <0.001 | <0.02 |

[a] μmoles glycerol/g wet weight/2 h
[b] Glucose-containing – glucose free medium

out whether the glucose potentiating effect on noradrenaline and dibutyryl cAMP induced lipolysis would occur simultaneously. Incubations were performed during 30, 60 and 120 min in medium containing either noradrenaline ($3\times10^{-}$ M) or dibutyryl cAMP ($10^{-3}$ M) and with and without glucose (2 mg/ml). The rate of lipolysis for each period (0–30 min, 30–60 min and 60–120 min) was calculated by subtracting the glycerol release during the previous period from the glycerol release observed during the period under consideration. Means of three experiments in which the rate of glycerol release was expressed ... min are presented in Fig. 4. It is observed in glucose free medium the noradrenaline induced lipolysis was significantly decreased after the first 30 min whereas the dibutyryl cAMP induced lipolysis was fairly constant during the whole incubation time.

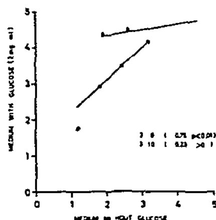



*Fig. 5.* Relationship between isopropylnoradrenaline (ISPNA) induced glycerol release in presence and absence of glucose. Values are expressed as μmoles glycerol/g wet weight/2 h incubation. ● $3\times10^{-7}$ M ISPNA ($N=11$, $Y=0.89X+1.32$). ▲ $3\times10^{-5}$ M ISPNA ($N=8$, $Y=0.17X+4.00$).

In order to find out whether the effect of glucose on noradrenaline induced lipolysis is due to a decreased potency of alpha adrenergic receptor or to an increased "response on stimulation" of beta adrenergic receptors, we studied the effect of glucose on lipolysis induced with an almost pure beta adrenergic stimulator, isopropylnoradrenaline. Table III shows that glucose significantly ($p<0.001$–$<0.02$) stimulates glycerol release produced by two different concentrations of isopropylnoradrenaline ($3\times10^{-7}$ and $3\times10^{-5}$ M). In separate experiments we have shown that this higher concentration of the drug ($3\times10^{-5}$ M) induces maximal stimulation of lipolysis in human adipose tissue. When the isopropylnoradrenaline induced glycerol release in glucose free media was plotted against the stimulated glycerol release in glucose-containing media, significant correlation was observed only for the lower dose of isopropylnoradrenaline ($p<0.01$) (Fig. 5). Slopes for both concentrations of isopropylnoradrenaline (Fig. 5) were significantly different from the slope showing the influence of glucose on dibutyryl cAMP induced lipolysis ($p<0.02$ and $<0.001$). As in the experiments with noradrenaline the stimulatory effect of glucose was more pronounced in tissues showing poor response to isopropylnoradrenaline, especially when the agent was added at the higher concentration ($3\times10^{-5}$ M).

In seven experiments we investigated ...

Table IV *Influence of glucose on gl)cerol release and on FFA concentration in medium and tissu during in vitro incubation of adipose tissue (Mean±S.E. N=7)*

Incubation conditions are given in Table I

| Glucose concentration | Basal conditions | | | NA 3 10 ⁵ M | | |
|---|---|---|---|---|---|---|
| | Gl)cerol[a] | Δ FFA in medium[b] | Δ FFA in tissue | Gl)cerol | _ FFA n medium | FFA in t e |
| | 0.51±0 07 | 0 66±0 12 | −0 45±0.-0 | 2 37±0.-4 | 4 1 ±0 6. | ± - |
| 2 mg ml | 0 80±0 08 | −0 11±0.-0 | −0 55±0 0 | 3 88±0.-6 | 4 15± ) ⁵ | – |
| Mean diff ± S.E.M | 0 .-8±0 07 | −0 77±0 12 | −0 10±0 14 | 1 52±0 27 | 0 0 ±0 4 | ± |
| p | <0 005 | <0 001 | >0 1 | <0 005 | 0 | |

[a] µmoles glycerol g wet weight/2 h
[b] µEq FFA g wet weight/2 h
[c] Glucose-containing – glucose-free medium

flu.nc. of glucose on the FFA changes in tissues and incubation media under basal conditions and in th pres.nc. of noradrenaline ($3\times10^{-5}$ M) (Table IV) In the presence of glucos the net releas of FFA into basal media was significantly decreas.d ($p<0\ 001$) whereas no changes in the FFA concentration of tissue were observed Opposi t results w re obtain d in the noradrenaline exp riments Glucose had no significant influence upon the n t releas of FFA but increased slightly ($p<0\ 05$) the tissu conc.ntration of FFA

Table V pr sents th results from exp riments on the influenc. of 2-deox, d g ucose on lipol) is in human omental adipos tissu. Add ion of 2 deoxy-*d* glucose inhibited signifi antl) the lipol)sis stimulated by noradrenalin isopropylnoradre naline and dibutyryl cAMP but did not alt r th basal gl)cerol releas

Table V *Influence of glucose and 2-deoxy-d glucose on basal and stimulated lipolysis in human omental tissue*

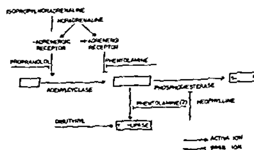
Incubation cond tions are given in Table I

| Addition to med um | Gl)cerol release[a] | | |
|---|---|---|---|
| | 0 | glucose 2 mg ml | 2-deoxy-*d*-glucose 2 mg ml |
| 0 | 0 34 | 0 63 | 0.35 |
| 0 | 0 25 | 0 41 | 0 37 |
| 0 | 0 38 | 1 12 | 0 45 |
| 0 | 0 23 | 0 34 | 0 16 |
| *l*-noradrenaline 3 10 ⁵ M | 2 04 | 2.32 | 0 89 |
| *l*-noradrenaline 3 10 ⁵ M | 1 24 | 2.96 | 0 61 |
| *l*-no adrenaline 3×10⁻⁵ M | 0 87 | 1 19 | 0 40 |
| *l*-isopropylno adrenaline 3×10⁻⁵ M | 2.28 | 3 65 | 1 56 |
| *l*-isopropylnoradrenaline 3×10⁻ M | 2.34 | 2.-6 | 0 89 |
| d butyryl cAMP 10⁻³ M | 1 64 | 1 50 | 0 56 |
| d butyryl cAMP 10⁻³ M | 4 16 | 4 72 | 1 78 |
| dibutyryl cAMP 10 ³ M | 6 13 | 7 28 | 1 75 |

[a] µmoles g wet we ght/2 h

## DISCUSSION

Recent in vitro studies in our laboratory are compatible with the hypothesis that lipol)sis in human adipose tissue is mediated by the accumulation of c)clic adenosine 3 5 monophosphate which would




*F g 6* Schematic picture of the me hanisms regulating lipolysis in human adipose tissue in vitro incl ding probable sites of action of ubstances w th agon antagonistic effect. See text for explanation.

Table VI *Effect of 5 AMP on theophylline induced changes in glycerol release and incorporation of glucose 1 $^{14}$C into lipids of human omental tissue in vitro*

| Exp no | Glycerol release[a] | | | | | Glucose-1 $^{14}$C incorporation into total tissue lipids[b] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Theophylline ($10^{-3}$ M) plus 5 AMP | | | | | Theophylline ($10^{-3}$ M) plus 5 AMP | | | |
| | Basal | (0) | ($10^{-5}$ M) | ($10^{-4}$ M) | ($10^{-3}$ M) | Basal | (0) | ($10^{-5}$ M) | ($10^{-4}$ M) | ($10^{-3}$ M) |
| 17 | 0 80 | 5 73 | — | — | 1 50 | 0 61 | 0 41 | — | — | 0 40 |
| 18 | 1 01 | 8 96 | 7 41 | 6 83 | 2 49 | 1 60 | 0 89 | 0 0 | 0 75 | 0 56 |
| 19 | 0 73 | 6 59 | 7 37 | 5 79 | 1 10 | 0 77 | 0 62 | 0 60 | 0 49 | 0 40 |

[a] μmoles g lipid 2 h
[b] μmoles glucose g lipid 2 h
Incubation conditions the same as in Table I

this substance was added at different concentrations to theophylline-containing medium (Table VI) No inhibition of the antilipogenic effect of theophylline was seen but rather a further decrease in the glucose utilization This was especially true for the higher concentration of 5 AMP ($10^{-3}$ M) which had a strong antilipolytic effect.

As shown in Table VII in which the results of thirteen experiments are summarized a considerable amount of FFA is re-esterified by human omental tissue incubated in the presence of glucose (2 mg ml) In fact the mean rate of re esterification of FFA exceeded the theoretical value of FFA production in tissue 0 80 × 3 les g lipid This could be explained by a cer net accumulation of partial glycerides The of noradrenaline (3 $10^{-5}$ M) to the -containing medium produced a fivefold increase in the glycerol release and a twofold rise in the rate of re-esterification Lipolysis and re esterification were strongly interrelated under basal conditions ($r$ 0 91 $p < 0 001$) as well as in the presence of noradrenaline ($r$ 0 80 $p < 0 001$) which is illustrated in Fig 2.

Table VII *Effect of noradrenaline on the balance between fatty acid and glycerol production in human omental tissue in vitro*

| | Basal | Noradrenaline |
|---|---|---|
| FFA net release | 0 72 ± 0 17 | 1 51 ± 0 31 |
| Net changes in tissue FFA | 0 03 ± 0 19 | 5 07 ± 0 49 |
| Glycerol release | 0 80 ± 0 07 | 4 49 ± 0 36 |
| Re-esterification of FFA | 3 10 ± 0 39 | 6 89 ± 1 1 |

Values given in μEq g lipid Mean and standard error f thirteen experiments. Tissue segments of total weight of approximately 00 mg incubated in 4 ml of Krebs-Henseleit bicarbonate buffer with 3 g 100 ml of bovine serum albumin and 2 mg ml of glucose Noradrenaline added at a concentration of 3 $10^{-5}$ M Incubations run in duplicate Re-esterification calculated as glycerol release 3 − net production of FFA in tissue plus medium (37)

Also in experiments in which lipolysis was accelerated by dibutyryl cAMP re-esterification was markedly stimulated (Table VIII) Within each single experiment the changes in lipolysis and re-esterification of FFA seem to run in parallel On the other hand theophylline produced a prominent decrease in the rate of re-esterification although the regular stimulation of lipolysis was achieved (Table VIII) Apparently these findings were in consonance with the aforementioned results on glucose utilization

## DISCUSSION

In consonance with a recent observation on human subcutaneous tissue (1) the present studies show that a considerable part of glucose 1 $^{14}$C incorporated into lipids in segments of human omental tissue is recovered as glyceride fatty acid By use of a similar method other authors (19) have demonstrated that labelled glucose fatty acid of subcutaneous adipose tissue averaged only 15 % of the total tissue lipid label Much lower figures for the synthesis of fatty acids have been observed in human isolated fat cells (16 17) and in 2 mm thick strips of human omental (14 23) as well as subcutaneous tissue (14 15) The rate of conversion of glucose into glyceride fatty acid is apparently lower in isolated fat cells than in segments of subcutaneous tissue (19) Other authors have reported that the conversion of glucose 1 $^{14}$C

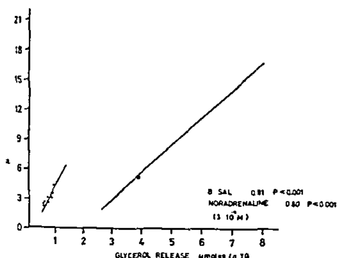



*Fig 2* Relationship between lipolysis nd re-esterification Incubation conditions the same as given in Table VII

$CO_2$ and total lipid was lower in needle iopsy specimens than in tissue segments of 20–100 mg weight (4) which is in agreement with revious studies on rat adipose tissue (22)

A detailed and recently reported study (18) to clarify to a great extent why so variable ults have been obtained by different authors. that study it was well demonstrated that pro- incubation of isolated human omental fat lls led to a relatively higher rate of the fatty synthesis than of the glyceride glycerol for tion Since w like Bjorntorp (1 2) use a cer tain time of preincubation when the metabolic ity of segments of tissue is examined this design of study might favor the fatty acid syn thesis

The lower figure for fatty acid synthesis re ported by some authors in human adipose tissue has forced some investigators to search for possi ble differences between the enzyme activities of human adipose tissue and rat epididymal fat pad Recently it has been reported that the concentra tion of enzymes required for fatty acid synthesis was considerably less in extracts prepared from human omental adipose tissue than from rat epidi dymal fat (31 32) In the light of the cited studies of Goldrick et al (18) it seems pertinent to study the changes in the activity of the citrate cleavage

Table VIII *Comparison of the effects of noradrenaline dibutyryl cAMP and theophylline on glycerol release re-esterification of FFA in human omental tissue in vitro*

| Exp no | Glycerol release | | | | Re-esterification[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | Basal | Noradrenaline (3 10-6 M) | Dibutyryl cAMP (3 × 10-4 M) | (10-3 M) | Basal | Noradrenaline (3 10-6 M) | Dibutyryl cAMP (3 10- M) | (10- M) |
| | 0 89 | 2 73 | 1 35 | 6 34 | 5 38 | 10 5- | 6 53 | 13 74 |
| | 0 89 | 4 1 | 1 84 | 4 54 | 3 41 | 4 57 | 4 31 | 4 30 |
| | 0 89 | 3 44 | 2 46 | 3 74 | - 52 | 6 63 | 4 70 | 6 44 |
| | Basal | Noradrenal ne (3 × 10- M) | Theophylline (10-3 M) | Noradrenal ne (3 10-6 M) plus theophyl l ne (10-3 M) | Basal | Noradrenaline (3 10-6 M) | Th ophyll ne (10- M) | Noradrenaline (3 10-6 M) plus theophyl l e (10- M) |
| -5 | 0 72 | 5 17 | 5 -2 | 6 22 | 3 05 | 9 30 | 3 01 | 2 43 |
| 26 | 0 99 | 2 36 | 6 48 | 11 02 | 5 20 | 7 73 | 3 41 | 6 03 |
| 27 | 0 56 | 2 63 | 4 00 | 6 44 | 2 38 | 3 13 | 1 18 | 1 16 |

a / mole g lip d/2 h
/ Eq FFA g l pid/2 h
Incubation cond t ons the same as in Table VII

enzyme in human adipose tissue during prolonged incubation before final conclusions can be drawn from these findings. When data from the studies on segments of adipose tissue are taken together there is little doubt that glucose is of importance not only for the esterification of FFA formed in or taken up into adipose tissue but contributes also to the de novo synthesis of long-chained fatty acids This is further shown from studies in which glucose load to the donors significantly stimulated fatty acid synthesis (5)

Like other authors we have observed a marked variability in the lipogenesis between adipose tissue removed from different donors (1 22) To some extent this interindividual variability might depend on the difference in size of the fat cells obtained from different donors since we have expressed the metabolic activities per lipid weight and not per cell number However from the data of Salans et al (29) it is obvious that the variation of for instance glucose oxidation expressed per cell number is almost as great as that when glucose oxidation is calculated per lipid weight In the present study the variation of the incorporation of glucose into fatty acids was less pronounced than the variation of the conversion of glucose into glyceride glycerol and the data on fatty acid synthesis are in general more consistent in this study This could be due to the fact that the lab l of glyceride glycerol represents not only triglyce but also partial glycerides

In almost each experiment noradrenaline and adrenaline added up to a concentration known to produce maximal lipolysis ($3 \times 10$ M) increased the utilization of glucose by omental tissue The incorporation of label was stimulated not only into glyceride glycerol but also and more consistently into glyceride fatty acid This observation differs from the only one hitherto reported (15) in which small strips of tissue were used and in which a decrease in the synthesis of fatty acids was demonstrated The discrepancy between these results might to some extent be explained by the difference in the handling of tissue as discussed in the foregoing.

As expected from the findings on the utilization of glucose noradrenaline significantly increased also the rate of re-esterification of FFA calculated according to the non isotopic balance method of Vaughan (37) This latter finding confirms the recent report of Bjorntorp et al (3) in omental tissue who previously observed also that the re esterification in human subcutaneous tissue is low (2) and not stimulated by noradrenaline (2) As previously discussed (2 3) the accuracy of the balance method may be questioned since the production of FFA and glycerol formed from partial glycerides preexisting in tissue before incubation or accumulating in tissue during incubation would result in an over and underestimation of re esterification respectively As in similar experiments in rat adipose tissue (30) the early observation (39) that injection of adrenaline in rabbit produced an accumulation of partial glycerides in adipose tissue would suggest that some accumulation of partial glycerides occurred in the present experiments This would mean that the present figure for re-esterification of FFA is at least no overestimation It has to be added that in the present study it was ascertained that no transformation of glucose directly to glycerol took place Previously it has also been shown (43) that no significant utilization of glycerol by omental tissue occurred

A number of observations in the present study strongly indicate that the increase in the incorporation of glucose into tissue lipids as well as the accelerated rate of the re-esterification of FFA are events secondary to an enhanced triglyceride breakdown This is supported first by the observation that all lipolytic agents except theophylline significantly increased the triglyceride synthesis determined by the isotopic method and/or by use of the balance method of Vaughan (37) This holds true for noradrenaline adrenaline isopropylnoradrenaline and dibutyryl cAMP In addition stimulation of noradrenaline induced lipolysis by phentolamine resulted in increased conversion of glucose into tissue lipid whereas inhibition by propranolol was concomitant with a suppression of glucose utilization Lastly high concentrations of phentolamine significantly inhibited lipolysis and lipogenesis induced either by noradrenaline or dibutyryl cAMP The only agent in these experiments which had a strong lipolytic effect but no stimulatory effect on triglyceride synthesis was theophylline which will be discussed later The intimate relationship between lipolysis and re-esterification of FFA under basal conditions as well as after noradrenaline gives support for a direct relationship between the process in omental tissue thus in similarity to findings in rat adipose

ssu. It has to b emphasized that the experi nts on rat adipose tissue in which a relationship ween lipolysis and glucose utilization has b en questioned (9) are not well understood. Thus it as shown in that study that propranolol did in bit the effect of adrenaline on the conversion of lucose to glyceride-glycerol and glyceride fatty cid, but did not block the oxidation of glucose. Our present findings are well in accordance with other data in rat adipose tissue (12, 26 36) from hi h it has been concluded that the elevated con ntration of FFA in stimulated tissue is respons ble for the changes in the synthesis of triglyce ides. Another factor of possible physiologic sig finance has been suggested by Blecher (6). He as demonstrated that dibutyryl cAMP as well as heophylline when added at concentrations which ncreased the release of glycerol produced a sig nifi ant decrease in the utilization of glucose by at adipose tissue. This was later (8) attributed to n inhibitory effect of cyclic AMP on one of the ADPH producing enzymes 6-phosphogluconate dehydrogenase of the hexose monophosphate hunt. Furthermore Bray (8) has suggested that he decrease in synthesis of fatty acids induced by epinephrine in rat adipose tissue (12) was due to accumulation of cyclic adenosine 3 5 monophos-ph te (3,5 AMP) with subsequent inhibition of the pentose cycle. Our experiments showed that in human omental adipose tissue catecholamines as well as dibutyryl cAMP stimulate fatty acid and glyceride glycerol synthesis which would sugg st that accumulation of free fatty acids in this tissue is accompanied by enhancement of both the glycolytic and the pentose phosphate pathway.

As in rat adipose tissue (6) theophylline was shown in the present study to inhibit the synthesis of triglycerides at least when a high concentration (10 M) of the agent was used. The absence of this inhibitory effect of theophylline in a con centration of 10 3 M by which lipolysis was significantly stimulated suggests that the effects of th agent on glucose utilization reflect two opposite effects 1) increase in the glucose utilization due to accelerated lipolysis and 2) decrease in the utilization of glucose above the level of glyceryl aldehyde phosphate by some other and more prominent mechanism. From the present study it is not possible to relate this second mechanism to the inhibitory effect of theophylline on the activity of phosphodiesterase and the subsequent decrease in the formation of 5 AMP since the addition of different concentrations of this agent had no influence upon the theophylline suppressed li pogenesis.

## REFERENCES


1. Bjorntorp P. The effect of n ulin in vitro on human adipose tissue from normal and d betic sub jects. Ac a med scand 181 389 196
2. — Lipid mobilization from human ubcutan ous adipose ti sue in vitro. A ta med scand 18 1 1967
3. Bjorntorp P. Karlsson, M & Hovden A. Quantita tive aspects of lipolysis and re e erif ati n n human adipose tissue in vitro. Acta med scand 18 89 1969
4. Bj rntorp P & Martinsson A. Con ersion of C glucose to carbon dioxide and lipids n human adip se tissue in vitro. Acta med. scand 181 3 9 1967
5. Bjorntorp P. S hersten T & Gottfries A. Effects of glucose infusions on adipose t ssue lipogenesis in man. Acta med. scand 183 6 1968
6. Blecher M. Evidence for the in ol ement of cyclic 3,5 adenosine monophosph te in glu se utilization by isolated rat epidid mal adipose cel s. Bio hem. biophys. Res Commun 7 560 1967
7. Bray G A. A simple efficient liquid c ntillator for countin aqueous solutions in a liquid scir llat on counter. Analyt Bio hem 1 79 1960
8. — Inhibition of glucose oxidation in adipos ti sue by dibutyryl adenosine 3 5 phospha e. L ch m bio-phys. Res. Commun 8 631 1967
9. — Effe ts of epinephrine corticotropin and thyrotropin on lipolysis and glu ose oxidation in rat ad pose tissue. J Lipid Res. 8 300 1967
10. Bray G A & Goodman H M. Eff cts of e ne phrine on glucos tran port and metabolt m in ad pose tissue of normal and hypothyroid rats. J Lipid Res. 9 714 1968
11. Brown, J L & John ton, J M. Radioassay of lipid components separated by thin layer chromatography. J Lipid Res. 3 480 196
12. Cahill G F Jr Leboeuf B & Flinn R B. Studies on rat adipose tissue in vitro VI Effect of epinephrine on glucose metabolism. J biol Chem 35 1 46 1960
13. Dole V P. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. J clin. In est 35 150 1956.
14. Fessler A & Beck J C. The effect of insulin on the metabolism of human adipose tissue in itro. Biochim. biophys. Acta (Amst.) 106 199 1965
15. Fessler A, Beck, J C & Rub n ten, D. Factors affecting l pid synthesis in human adipose tissue in vitro. Metabolism 16 438 1967
16. Galton D J. Lipogenesis in human adipose tissue. J Lipid Res 9 19 1968
17. Goldrick, R B. Effects of insulin on glucose metabolism in isolated human fat cells. J Lip d Res. 8 581 1967

18. Goodridge, R. B. Ashe, B. C. E. & Lloyd, M. L. Effects of prolonged incubation and cell concentration on lipogenesis from glucose in isolated human omental fat cells. J Lipid Res. 10 213 1969
19. Gries, F. A. & Steinke, J. Comparative effects of insulin on adipose tissue segments and isolated fat cells of rat and man. J clin. Invest. 46 1413 1967
20. Hamosh, M., Hamosh, P., Bar Maor, J. A. & Cohen, H. Fatty acid metabolism by human adipose tissues. J clin. Invest. 42 1648 1963
21. Himms-Hagen, J. Sympathetic regulation of metabolism. Pharmacol. Rev. 19 367 1967
22. Hirsch, J. & Goldrick, R. B. Serial studies on the metabolism of human adipose tissue. I. Lipogenesis and free fatty acid uptake and release in small aspirated samples of subcutaneous fat. J clin. Invest. 43 1776 1964
23. Kahlenberg, A., Rubinger, J. & Kalant, N. Differences in the glucose metabolism and insulin responsiveness of rat and human adipose tissue. Canad. J Biochem. 44 645 1966
24. Larsen, J. A. Elimination of glycerol as a measure of the hepatic blood flow in the cat. Acta physiol. scand. 57 224 1963
25. Leboeuf, B. Flinn, R. B. & Cahill, G. F., Jr. Effect of epinephrine on glucose uptake and glycerol release by adipose tissue in vitro. Proc. Soc. exp. Biol. Med. N.Y. 102 527 1959
26. I ... S ... R. ... & B... R. H. Effects of ... and ... on the metabolism of rat adipose tissue. J biol. Chem. 235 ... 19...
27. ... Standard ... Co. ... Statistical ... 
28. ... D. A. D. Gi... M. Comparative studies on ... adipose tissue. In Advances in ... & Paoletti, A. D. ... ... Academic Press, London and New York.
Salans ... J. L. & Hirsch, J. The ... of ... and adipose tissue insulin sensitivity in ... of human obesity. J ... 19..
30. Sa... R. P. ... F. ... Park, T. ... & Clary T. R. Effect of ACTH on FFA release and glycerol content ... tissue. Ann. N.Y. Acad. Sci. 1 ... 
31. Shrago, E., Glennon, J. A. & Gordon, E. S. Studies on enzyme concentration and ... in human liver and adipose tissue ... Endocr. 27 679 1967
32. Shrago, E., Spennetta, T. & Gordon, E. S. Fatty acid synthesis in human adipose tissue. J biol. Chem. 244 2761 1969
33. Snedecor, G. W. Statistical methods. Iowa State College Press, Iowa 1957
34. Stahl, E. Thin layer chromatography ... Springer Berlin 1965
35. Trout, D. L., Estes, E. H. & Friedberg, S. J. Titration of free fatty acids of plasma: a study of current methods and a new modification. J Lipid Res. 1 199 1960.
36. Vaughan, M. Effect of hormones on glucose metabolism in adipose tissue. J biol. Chem. 236 2196 1961.
37. — The production and release of glycerol by adipose tissue incubated in vitro. J biol. Chem. 237 3354 1962.
38. Verner, J. V., Jr. Blackard, W. G. & Engel, F. L. Some factors modifying the actions of hormones on glucose uptake by adipose tissue in vitro. Endocrinology 70 420 1962.
39. Wadström, L. B. Lipolytic effect of the injection of adrenaline on fat depots. Nature 179 259 1957
40. Wieland, O. Eine enzymatische Methode zur Bestimmung von Glycerin. Biochem. Z. 329 313 1957
41. Östman, J. A procedure for in vitro studies on fatty acid metabolism by human subcutaneous adipose tissue. Acta med. scand. 177 183 1965
42. — Lipolysis. Species differences: human and experimental animals? In: Protein and peptide hormones (ed. M. Margoulies) vol. 3 p. 741. Excerpta Medica Foundation, Amsterdam 1969
43. — Unpublished observations.
44. Östman, J. & Efendic, S. Catecholamines and metabolism of human adipose tissue. II. Effect of isopropylnoradrenaline and adrenergic blocking agents on lipolysis in human omental adipose tissue in vitro. Acta med. scand. 187 471 1970.
45. Östman, J., Efendic, S. & Arner, P. Catecholamines and metabolism of human adipose tissue. I. Comparison between in vitro effects of noradrenaline, adrenaline and theophylline on lipolysis in omental adipose tissue. Acta med. scand. 186 241 1969

Acta med scand Vol 187 pp 503-507 1970

# INFLUENCE OF PROSTAGLANDIN $E_1$ ON LIPOLYSIS INDUCED BY NORADRENALINE ISOPROPYLNORADRENALINE THEOPHYLLINE AND DIBUTYRYL cAMP IN HUMAN OMENTAL ADIPOSE TISSUE IN VITRO

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*Abstract* The antilipolytic effect of prostaglandin $E_1$ (PGE) has been studied in human omental adipose tissue in vitro in order to explore the site of its action. Samples of omental tissue obtained during surgery were incubated in Krebs-Henseleit bicarbonate buffer containing bovine albumin and glucose Glycerol release was taken as an index of lipolysis. $PGE_1$ had no influence on either basal lipolysis or on lipolysis induced with N 2 0-dibutyryl AMP but significantly decreased the glycerol release induced by theophylline $PGE_1$ inhibited lipolysis induced by isopropylnoradrenaline more effectively than that by noradrenaline This difference was eliminated by the addition of the alpha adrenergic blocking agent phentolamine to incubation medium containing noradrenaline Previously we have shown that phentolamine regularly potentiated the lipolysis induced by noradrenaline indicating that the alpha adrenergic effect of noradrenaline in human adipose tissue has an inhibiting influence on lipolysis. With this background the present findings were interpreted as indicating that $PGE_1$ inhibits the alpha as well as the beta adrenergic effect of noradrenaline The net effect of $PGE_1$ on lipolysis in human omental adipose tissue in vitro seems to depend on the activity of these receptors.

It has been demonstrated that prostaglandins especially $PGE_1$ inhibit lipolysis induced by noradrenaline or other hormones in rat adipose tissue in vitro (1 12, 13) Similar findings have been reported from studies on human adipose tissue in vitro (2 5) Controversial opinions exist about the mechanism behind the antilipolytic effect of prostaglandins

In the present experiments the effect of $PGE_1$ was studied on the lipolysis induced by noradrenaline isopropylnoradrenaline theophylline and dibutyryl cAMP in human omental tissue in an attempt to localize the site of its inhibitory effect on lipolysis.

## MATERIAL AND METHODS

Adipose tissue was obtained from patients undergoing cholecystectomy The patients fasted overnight and were premedicated before operation with atropine and Pethidin® (Aco Stockholm Sweden) Anesthesia was induced with short acting barbiturate (Narkotal® Astra Sodertalje Sweden) and continued with Halothane® (ICI England) $N_2O$ and $O_2$ in combination with suxomethonium chloride (Celocurin® Vitrum Stockholm Sweden) Samples of adipose tissue were obtained from the major omentum 30-50 min after the start of operation During this time saline but not glucose was infused intravenously The adipose tissue was immediately placed into Krebs-Henseleit bicarbonate buffer (pH 7 4) containing albumin (1%) kept at 37 C and divided into pieces weighing approximately 50-100 mg. After 60 min preincubation the tissue sections were transferred into Packard plastic vials containing 3 ml of buffer (pH 7 4) with the addition of 3 g 100 ml of bovine serum albumin (Armour Pharm. Co Eastbourne England, Lot number NH 0870) and glucose (100 mg 100 ml) About 200 mg of adipose tissue was incubated in each vessel After two hours of incubation aliquots of medium were removed for glycerol determination according to Wieland (15) as modified by Larsen (7) Statistical calculations have been made according to Snedecor (11) Agents added in vitro were noradrenaline bitartrate (supplied by Astra, Sodertalje Sweden) *l* isopropylnoradrenaline-*d*-bitartrate dihydrate (supplied by Dr F P Luduena from Sterling Winthrop Research Institute) phentolamine HCL as a pure substance (supplied by Ciba AB Stockholm Sweden) prostaglandin $E_1$ (supplied by Dr E Änggård, Karolinska Institute Stockholm, Sweden) Noradrenaline isopropylnoradrenaline and phentolamine were dissolved in distilled water 0 1 ml of this was added to incubation medium. Theophylline was directly dissolved in the albumin-containing buffer $PGE_1$ was dissolved in absolute alcohol and diluted in bicarbonate buffer The final concentration of ethanol in a medium with the highest concentration of $PGE_1$ ($1 \mu g/ml$) was about 0.008 % From other experiments it was ascerta[illegible] th

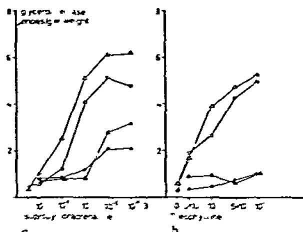


*Fig 1 (a)* Effect of $PGE_1$ (1 µg/ml) on isopropylnoradrenaline-induced lipolysis. Human omental adipose tissue (approx. 200 mg) was incubated for 2 hours in Krebs-Henseleit bicarbonate buffer with 3% bovine albumin and 1 mg/ml glucose added. Parallel incubations with isopropylnoradrenaline (○—○ △—△) and with isopropylnoradrenaline plus $PGE_1$ (●—● ▲—▲) were run in quadruplicate.

(b) Effect of $PGE_1$ (1 µg/ml) on theophylline-induced lipolysis. Theophylline ○—○ △—△. Theophylline plus $PGE_1$ ●—● ▲—▲. Each point represents mean of four incubations. (Incubation conditions given in *a*)

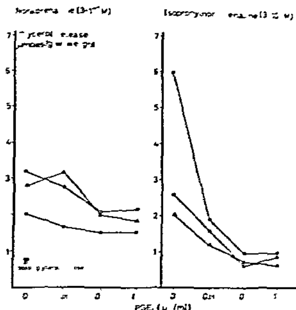


*Fig 2* Comparison between effects of different concentrations of $PGE_1$ on noradrenaline and isopropylnoradrenaline induced lipolysis. Each point represents mean of three incubations. (Incubation conditions given in Fig. 1 *a*)

centration of ethanol was far less than those producing an increase in the glycerol release from human adipose tissue.

## RESULTS

[illegible] first series of experiments were designed to [illegible] the influence of $PGE_1$ (1 µg/ml) on lipolysis induced with either the beta adrenergic stimulator isopropylnoradrenaline or with theophylline, which is regarded as an inhibitor of the phosphodiesterase activity.

It is shown in Fig. 1 *a* that $PGE_1$ almost completely blocked the lipolytic effect of isopropylnoradrenaline added in a concentration of $3 \times 10^{-7}$ M or lower. The decrease in lipolysis was

*Table I. Comparison between the effect of prostaglandin $E_1$ (1 µg/ml) on noradrenaline (NA), noradrenaline plus phentolamine (NA + Pr), isopropylnoradrenaline (ISPNA) and dibutyryl cAMP (dAMP) induced lipolysis*

| Experiment | Addition to medium[a]: 0 | PGE₁ | NA 6×10⁻⁷ M | PGE₁ | NA 3×10⁻⁶ M | PGE₁ | NA 6×10⁻⁷ M + Ph[b] | PGE₁ | NA 3×10⁻⁶ M + Ph[b] | PGE₁ | ISPNA 10⁻⁶ M | PGE₁ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.50 | 0.69 | [illegible] | [illegible] | 4.00 | [illegible] | 3.95 | [illegible] | 6.15 | [illegible] | [illegible] | 0.84 |
| 2 | 0.59 | 1.06 | [illegible] | [illegible] | 4.60 | 3.81 | 3.45 | 0.82 | 5.62 | 3.37 | [illegible] | 1.18 |
| 3 | 0.61 | 0.68 | [illegible] | [illegible] | 3.68 | 3.19 | 2.91 | 1.06 | 4.20 | 2.13 | 1.56 | 0.48 |
| 4 | 0.60 | 0.42 | [illegible] | [illegible] | 4.08 | 2.20 | 2.89 | 0.41 | 3.33 | 2.07 | 1.53 | 0.54 |
| 5 | 0.31 | 0.39 | [illegible] | [illegible] | 2.60 | [illegible] | [illegible] | 0.45 | 6.40 | 2.84 | 2.22 | 0.56 |
| 6 | 0.57 | 0.50 | – | 1.03 | 1.95 | 1.31 | [illegible] | 0.87 | [illegible] | 1.31 | [illegible] | [illegible] |
| M ± S.E. | 0.53 ± 0.04 | [illegible] ± 0.10 | [illegible] | [illegible] ± 0.10 | [illegible] ± 0.40 | [illegible] ± 0.40 | [illegible] ± 0.27 | [illegible] ± 0.10 | 5.02 ± 0.50 | 2.50 ± 0.33 | 1.69 ± 0.15 | [illegible] ± 0.10 |

[a] Values expressed as µmoles glycerol/g wet weight/2 h.
[b] Phentolamine 50 µg/ml. Incubation conditions given in Fig. 1

about 60% when isopropylnoradrenaline was present in higher concentrations ($3 \times 10^{-6}$ M and $3 \times 10^{-5}$ M) which are known to induce maximal lipolytic response In both experiments $PGE_1$ significantly decreased the maximal lipolytic effect of isopropylnoradrenaline This type of modification of the dose response curve speaks in favour of a non-competitive antagonism Fig 1 *b* depicts dose response curves for glycerol release with theophylline alone and with theophylline plus $PGE_1$ in adipose tissue from two other subjects It is observed that $PGE_1$ almost abolished the lipolytic effect of all concentrations of theophylline As a consequence of the limited solubility of theophylline in the incubation medium it was not possible to study the interaction between $PGE_1$ and higher concentrations of theophylline In the present studies $PGE_1$ apparently inhibited the theophylline induced lipolysis in a non-competitive way

Fig 2 presents three experiments in which the effects of different concentrations of $PGE_1$ on lipolysis induced with either isopropylnoradrenaline ($3 \times 10^{-8}$ M) or noradrenaline ($3 \times 10$ M) were compared These concentrations of isopropylnoradrenaline and noradrenaline were chosen because they usually produce similar lipolytic response in human omental adipose tissue It is seen that $PGE_1$ at each concentration more strongly inhibited the isopropylnoradrenaline induced than the noradrenaline induced lipolysis In fact $PGE_1$ in a concentration of 0 1 $\mu$g/ml blocked completely the isopropylnoradrenaline produced glycerol release whereas the noradrenaline response was reduced by only 30 %

These data could be explained by the assumption that $PGE_1$ inhibits beta adrenergic as well as alpha adrenergic responses To test this hypothesis the effect of $PGE_1$ was studied in experiments in which the alpha adrenergic effect of noradrenaline was blocked by phentolamine $PGE_1$ was used in a concentration of 1 $\mu$g ml in these six experiments which included also studies on its effect on basal and on dibutyryl cAMP induced lipolysis Data of individual experiments are given in Table I No significant influence of $PGE_1$ on basal lipolysis was demonstrated Only in one experiment was a marked stimulation of basal lipolysis by $PGE_1$ observed (experiment no 2) whereas in other experiments either slight inhibition or stimulation occurred $PGE_1$ had no effect on the dibutyryl cAMP induced lipolysis On the other hand $PGE_1$ blocked noradrenaline as well as isopropylnoradrenaline induced lipolysis but as in previous experiments the antilipolytic effect was more prominent on the isopropylnoradrenaline induced glycerol release Lipolysis accelerated by low concentration of noradrenaline ($6 \times 10$ M) was completely blocked whereas the effect of high concentration of noradrenaline ($3 \times 10^{-5}$ M) was reduced on average by 19 % (+3 to −46 ) $PGE_1$ completely suppressed the lipolytic effect of low concentrations of isopropylnoradrenaline ($3 \times 10^{-9}$ M and $3 \times 10^{-8}$ M) whereas glycerol release produced by $3 \times 10$ M of isopropylnoradrenaline was decreased by an average of 41 % (−25° to −65 %) The more prominent antilipolytic effect of $PGE_1$ on isopropylnoradrenaline than on noradrenaline induced lipolysis was found not only when equipotent concentrations were compared but also when equimolar concentrations of the agents were taken into consideration

It can also be seen from Table I that phentolamine (50 $\mu$g/ml) in five experiments increased lipolysis induced with either low or high concentration of noradrenaline In one experiment (no 4) the stimulatory effect of phentolamine was achieved only in tissues incubated with low concentration of noradrenaline In all experiments $PGE_1$ had a strong antilipolytic effect when added to medium containing both noradrenaline and phentolamine Thus lipolysis induced with $6 \times 10^{-7}$ M of noradrenaline plus phentola as

| PNA $10^{-}$ M | | ISPNA $10^{-8}$ M | | dAMP $10^{-3}$ M | |
|---|---|---|---|---|---|
| | $PGE_1$ | | $PGE_1$ | | PGE |
| 14 | 0 76 | 6 17 | 4 35 | 4 17 | 4 48 |
| 8 | 0 98 | 5 87 | 4 39 | 7 71 | 8 23 |
| 7 | 0 63 | 4 44 | 2 38 | 2 95 | 2 99 |
| 05 | 0 54 | 5 11 | 2 71 | 3 87 | 4 31 |
| 00 | 0 70 | 6 93 | 4 48 | 7 33 | 7 69 |
| 79 | 0 76 | 4 77 | 1 78 | 4 06 | 3 73 |
| 32± 9 | 0 73± 0 05 | 5 55± 0 38 | 3 35± 0 49 | 5 02± 0 81 | 5 23± 0 89 |

totally suppressed, and the lipolytic effect of $3 \times 10^{-6}$ M of noradrenaline plus phentolamine was reduced by 50%. $PGE_1$ exerted similar antilipolytic effects on lipolysis stimulated by noradrenaline plus phentolamine as on lipolysis produced by isopropylnoradrenaline. This is true whether the comparison was made on the basis of molar concentrations ($3 \times 10^{-6}$ M of noradrenaline plus phentolamine and $3 \times 10^{-6}$ M of isopropylnoradrenaline) or of equipotent concentrations ($6 \times 10^{-7}$ M of noradrenaline plus phentolamine and $3 \times 10^{-8}$ M of isopropylnoradrenaline).

## DISCUSSION

It is well established that lipolysis in rat adipose tissue is controlled by the tissue level of cyclic AMP which activates lipase. Cyclic AMP is generated by means of the adenyl cyclase system and destroyed by the phosphodiesterase activity. Agents which either stimulate the activity of adenyl cyclase or inhibit the activity of phosphodiesterase are known to increase the tissue level of cyclic AMP and thus activate the triglyceride-splitting lipase (4). Recent studies indicate that similar mechanisms may well operate in human adipose tissue as well (16, 17).

Prostaglandin $E_1$, which inhibits basal and stim lipolysis in rat adipose tissue, is known to the level of cyclic AMP (3). In addition, been shown that PGE has no inhibitory on lipolysis stimulated by dibutyryl cAMP (14). The mechanism by which $PGE_1$ decreases cyclic AMP levels is still not known. Mühlbachova et al. (8) and Paoletti et al. (10) have shown that $PGE_1$ antagonized theophylline induced lipolysis in a competitive way, whereas the interaction between $PGE_1$ and noradrenaline was of a non-competitive nature. From these data these authors suggest that prostaglandins decrease the level of cyclic AMP by stimulating the phosphodiesterase activity. On the other hand Steinberg and Vaughan (14) have reported that $PGE_1$ did not inhibit the lipolytic effect of exogenous cyclic AMP, which indicated that $PGE_1$ inhibits adenyl cyclase in adipose tissue, as has been proposed by Orloff et al. (9). Our results would indicate that the antilipolytic effect of $PGE_1$ in human adipose tissue is localized at the level of adenyl cyclase, since we have demonstrated that: (*a*) $PGE_1$ had no effect on dibutyryl-cAMP induced lipolysis and (*b*) $PGE_1$ blocked more effectively isopropylnoradrenaline than noradrenaline induced lipolysis.

We have recently shown that in human adipose tissue the alpha adrenergic blocking agent phentolamine in low concentrations (0.05–100 $\mu$g/ml) stimulated noradrenaline induced lipolysis, and had no influence on isopropylnoradrenaline and theophylline effects (16). These data have been interpreted as indicating that in human adipose tissue there exist alpha- as well as beta-adrenergic receptors with opposite effects on lipolysis. Stimulation of alpha adrenergic receptors would then be concomitant with inhibition of lipolysis, whereas beta adrenergic receptors would accelerate lipolysis. The quantitative difference between the inhibitory effects of $PGE_1$ on noradrenaline and isopropylnoradrenaline induced lipolysis is explained if it *is agreed that $PGE_1$ inhibits the activation of both* alpha- and beta-adrenergic receptors. Noradrenaline stimulates both alpha- and beta-adrenergic receptors. In the light of our hypothesis, $PGE_1$ would simultaneously exert two opposite effects on noradrenaline induced lipolysis: an inhibitory effect due to inhibition of beta adrenergic responses, and a stimulatory effect due to inhibition of alpha adrenergic responses.

*l*-isopropylnoradrenaline is an almost pure beta-adrenergic stimulator. Therefore in the case of isopropylnoradrenaline induced lipolysis only the inhibitory effect of $PGE_1$ on beta-adrenergic responses would be apparent. Further evidence that *the difference in the action of $PGE_1$ on noradren*aline and isopropylnoradrenaline induced lipolysis lies in the alpha-adrenergic response induced by noradrenaline is furnished by the observation that the effect of $PGE_1$ on lipolysis induced by noradrenaline plus phentolamine is equal to that induced by isopropylnoradrenaline.

Recently Carlson et al. (6) demonstrated that $PGE_1$ increased the arterial plasma levels of FFA in fasting human subjects. By turnover rate studies it was shown that the rise in FFA concentration was caused by an increased mobilization of FFA into blood and not by reduced efflux of FFA from blood. One possible explanation of the different effect of $PGE_1$ on lipolysis in vivo and in vitro would be that $PGE_1$ under these in vivo conditions, for some unknown reason blocks the alpha-adrenergic receptors more effectively than the beta-adrenergic receptors.

## ACKNOWLEDGEMENTS

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## REFERENCES

1 Bergstrom, S & Carlson, L. A Influence of the nutritional state on the inhibition of lipolysis in adipose tissue by prostaglandin $E_1$ and nicotinic acid Acta physiol scand. 65 383 1965
2 — Inhibitory action of prostaglandin $E_1$ on the mobilization of free fatty acids and glycerol from human adipos tissue in vitro Acta physiol scand 63 195 1965
3 Butcher R W & Baird, C E Effects of prostaglandins on adenosine 3 ,5 monophosphate levels in fat and other tissues. J biol Chem. 243 1713 1968
4 Butcher R. W Baird C E & Sutherland E W Effects of lipolytic and antilipolytic substances on adenosine 3 5 monophosphate levels in isolated fat cells. J biol. Chem. 243 1705 1968
5 Carlson L. A. & Hallberg, D Basal lipolysis and effects of norepinephrine and prostaglandin $E_1$ on lipolysis in human subcutaneous and omental adipose tissue J Lab clin. Med 71 368 1968
6 Carlson, L. A. Ekelund, L. G & Oro L. Clinical and metabolic effects of different doses of prostaglandin E in man. Acta med. scand. 183 423 1968
7 Larsen, J A. Elimination of glycerol as a measure of the hepatic blood flow in the cat. Acta physiol scand 57 ?4 1963
8 Muhlbachova E. Sólyom A & Puglisi, L. Investigations on the mechanism of the prostaglandin $E_1$ antagonism to norepinephrine and theophylline induced lipolysis. Europe J Pharmacol 1 321 1967
9 Orloff J Handler S & Bergstrom, S Effect of prostaglandin ($PGE_1$) on the permeability response of the toad bladder to vasopressin theophylline and adenosine 3 ,5 monophosphate Nature 205 397 1965
10 Paoletti, R., Lentati, R. L. & Korolkiewicz, Z. Pharmacological investigations on the prostaglandin $E_1$ effect on lipolysis In Proceedings of the Second Nobel Symposium (ed S Bergstrom & B Samuelsson) p 147 Interscience Publ New York 1967
11 Snedecor G S Statistical methods Iowa State College Press, Iowa 1957
12 Steinberg, D Vaughan, M Nestel P J & Bergstrom, S Effects of prostaglandin E opposing those of catecholamine on blood pressure and on triglyceride breakdown in adipose tissue Bio hem. Pharmacol 12 764 1963
13 Steinberg, D Vaughan M Nestel P J Strand O & Bergstrom, S Effects of the prostaglandins on hormone induced mobilization of free fatty acids. J clin. Invest. 43 1533 1964
14 Steinberg, D & Vaughan M In vitro and in vivo effects of prostaglandins on free fatty acid metabolism In Proceedings of the Second Nobel Symposium (ed S Bergstrom & B Samuelsson) p 109 Interscience Publ New York 1967
15 Wieland O Eine enzymatische Methode zur Bestimmung von Glycerin. Biochem Z. 3 9 313 1957
16 Östman, J & Efendic S Catecholamines and metabolism of human adipose tissue II. Effects of isopropylnoradrenaline and adrenergic blocking agents on lipolysis in human omental adipose tissue in vitro Acta med scand. 187 471 19 0
17 Östman J Efendic, S & Arner P Catecholamines and metabolism of human adipose tissue I. Comparison between in vitro effects of noradrenaline adrenaline and theophylline on lipolysis in omental adipose tissue Acta med scand 186 41 1969

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# HEMODYNAMIC CHANGES IN LONG TERM DIURETIC THERAPY OF ESSENTIAL HYPERTENSION

*A Comparative Study of Chlorthalidone Polythiazide and Hydrochlorothiazide*

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*Abstract* Thirty-eight men with untreated essential hypertension in WHO stage I or II, aged 19–57 years, all working, have been studied ambulatorily Oxygen consumption, heart rate cardiac output (Cardiogreen) and intra arterial brachial pressure were recorded at rest in supine sitting and standing position and during steady state work. At rest plasma volume and serum electrolytes were measured. The subjects were divided into four groups I untreated 7 II chlorthalidone 100 mg every second day 9 III polythiazide 1 mg every second day 7 and IV hydrochlorothiazide 50 mg twice daily 15

The subjects were re studied after 8–12 months. In the *untreated* group none of the results at the second examination were significantly different from those at the first. In the *treated* groups the causal resting blood pressure and intra arterial pressure at rest and during work were significantly lower after treatment than before At rest when sitting, the mean arterial pressure was reduced 21 15 and 18% in groups II, III and IV and during work a little less Serum K dropped significantly in all groups, the mean drop being about 0 7 mEq/l in all groups Plasma volume (hydrochlorothiazide group only) was 0 4 l lower after therapy but the difference was not significant. In the *thiazide* groups the pressure drop was usually associated with a drop in peripheral resistance both at rest and during exercise In the *chlorothalidone* group the pressure drop was usually caused by a reduction in cardiac index, with little or no effect on the peripheral resistance

Several studies have shown that the basic hemodynamic disorder in the majority of subjects with essential hypertension after the age of 35 is an elevated peripheral vascular resistance (survey of literature in ref 11) The cardiac output is normal or reduced From a physiological point of view antihypertensive drugs should lower the arterial pressure in such patients by reduction of the resistance and not by reduction of the cardiac output

The hemodynamic effects of the thiazides and chlorthalidone have been well studied in short time experiments (1 4 5 6 8 9 12 13 14 15) but observations of their long term effect which seems to be different from the acute are rather scanty (3) Such studies could be of practical importance because if these drugs reduce the elevated peripheral resistance at rest and during exercise it would seem logical to use them prophylactically also in patients with moderate hypertension This is a report of a long term study of three oral diuretics widely used in treatment of hypertension today chlorthalidone polythiazide and hydrochlorothiazide

## MATERIAL

The study includes 38 men, age 19–57 years, with untreated essential hypertension but no other diseases. Secondary hypertension was excluded by the usual routine procedures (11) Table I shows the subjects divided into four groups according to the therapy given. Group I did not receive any treatment, and includes mostly younger patients with so slight hypertension at rest and during exercise that antihypertensive therapy was not started.

Groups II III and IV are rather similar although they were not matched. Practically all patients were in WHO s stage I one or two in each group in stage II (due to retinal changes) The habitual physical activity in the groups was about the same The known duration of hypertension was somewhat longer in group II

## METHODS

The subjects were studied hemodynamically at rest supine sitting and standing, and during bicycling in steady state at 300 600 and 900 kpm/min. Oxygen consumption, intraarterial pressure (brachial artery) heart rate and cardiac output (Cardiogreen) were measured in dupli

Table I *Age weight body surface area (BSA) physical activity hypertensive stage and known duration of hypertension in the subjects studied*

| Patient group | n | Age (y) Mean SD | Weight (kg) Mean SD | BSA (m²) Mean SD | Physical activity L | M | H | WHO stage I | II | Known duration of hypertension Mean | > 5 y | > 10 y |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I Untreated | 7 | 32.9<br>10 7 | 78 9<br>12.5 | 1 96<br>0 17 | 1 | 4 | 2 | 7 | 0 | 5 9 | 3 | 2 |
| II Chlorthalidone | 9 | 48 0<br>2.9 | 70 8<br>9 2 | 1 85<br>0 15 | 2 | 5 | 2 | 8 | 1 | 11 7 | 7 | 5 |
| III Polythiazide | 7 | 45 7<br>7 0 | 74 3<br>4 5 | 1 90<br>0 07 | 2 | 5 | 0 | 6 | 1 | 6 0 | 5 | 2 |
| IV Hydrochlorothiazide | 15 | 43 3<br>9 8 | 77 0<br>6 9 | 1 94<br>0 11 | 3 | 11 | 1 | 13 | 2 | 6 3 | 7 | 3 |

L = low M = medium H = high

in each situation The methods have been described in detail previously (11) In addition serum electrolytes were studied and in groups I and IV the plasma volume (Evans Blue)

All studies were done ambulatory After an observation period of 8-12 months the hemodynamic study was repeated In this period the patients received the following therapy group II chlorthalidone (Hygroton®) 100 mg every second day group III polythiazide (Renese®) 1 mg every second day group IV hydrochlorothiazide (Dichlotride®) 50 mg twice daily The subjects were instructed to eat one orange daily in addition to their usual unrestricted diet. Potassium preparations were given if serum K dropped to 3 0 mEq/l or less

The difference between the results of the first and the second study was tested by Student s t test

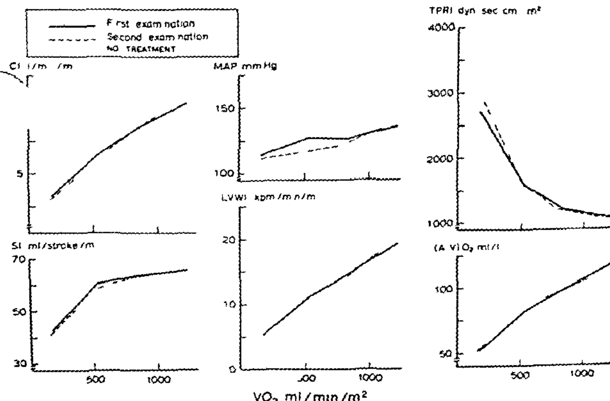



*Fig 1* Cardiac index (CI) mean arterial pressure (MAP) total peripheral resistance index (TPRI) stroke index (SI) left ventricular work index (LVWI) and arterio venous difference (A-V) O in relation to the gen consumption at first and second examination Untreated group (mean values)

## RESULTS

### A *Untreated Group*

There were no significant changes in casual blood pressure plasma volume serum electrolytes or hemodynamic parameters either at rest or during exercise as seen from Tables II–VII and from Fig 1 The oxygen consumption (VO ) was lower at the second study the difference being almost significant.

### B *Treated Groups*

*Th casual blood pressure*

The sitting casual blood pressure taken at the last visit before each of the hemodynamic studies dropped significantly in all groups as seen in Table II

*The plasma volume*

Th plasma volume (measured in the hydrochlorothiazide group only) dropped in all but two of 15 subjects the mean drop being 0 239 l or 7 % (Table III)

*The serum electrolytes*

Serum Na showed no significant changes (Table III) Serum K dropped significantly the mean drop being about 0 7 mEq/l in each group Serum K below 3 0 mEq/l was observed in one pati nt in groups II and III and in two patients in group IV Serum-Cl dropped in all groups the difference being significant in groups II and IV

Table II *Casual blood pressure at first (1) and second (2) examination (before and after therapy)*

The asterisks show the significance of the d fference between the results before and after the therapy

| Group | *n* | | Casual BP (mm Hg) SAP 1 | SAP 2 | DAP 1 | DAP 2 |
|---|---|---|---|---|---|---|
| I | 7 | Mean | 160 7 | 157 8 | 101 4 | 99 3 |
| | | S D | 8 4 | 11 1 | 5 6 | 7 3 |
| II | 9 | Mean | 178 8 | 136 7 | 116 7 | 97 2 |
| | | S D | 14 3 | 6 1 | 6 1 | 6 2 |
| III | 7 | Mean | 176 4 | 144 3 | 117 1 | 97 1 |
| | | S D | 7 5 | 6 1 | 5 7 | 4 9 |
| IV | 15 | Mean | 180 0 | 140 7 | 113 0 | 95 0 |
| | | S D | 18 4 | 10 3 | 11 9 | 7 3 |

SAP systolic arterial pressure DAP diastolic arterial pressure = 0 01 *P* 0 05 0 001 *P* 0 01 = *P* 0 001

*The oxygen consumption* (VO_)

At rest the oxygen consumption was slightly lower at the second examination (Table IV) The same tendency was seen also during work in groups II and III but in the largest group (IV) the mean values before and after therapy were almost identical

*The arterial pressure*

The systolic (SAP) diastolic (DAP) and mean arterial pressure (MAP) were reduced significantly

Table III *Plasma volume and serum electrolytes at first (1) and second (2) examination*

| Group | *n* | | Plasma volume (l) 1 | Plasma volume (l) 2 | Na (mEq/l) 1 | Na (mEq/l) 2 | K (mEq/l) 1 | K (mEq/l) 2 | Cl (mEq/l) 1 | Cl (mEq/l) 2 |
|---|---|---|---|---|---|---|---|---|---|---|
| I | 7 | Mean | 3 490 | 3 495 | 140 7 | 138 4 | 4 07 | 3 90 | 104 0 | 105 0 |
| | | S D | 0 55 | 0 53 | 3 2 | 3 5 | 0 26 | 0 19 | 2 4 | 1 7 |
| II | 9 | Mean | | | 139 1 | 135 [illegible] | 4 23 | 3 56 | 104 4 | 99 3 |
| | | S D | | | 3 4 | 5 8 | 0 [illegible]8 | 0 33 | 2 9 | 3 2 |
| III | 7 | Mean | | | 138 7 | 133 8 | 4 [illegible]5 | 3 47 | 105 0 | 96 1 |
| | | S D | | | 4 1 | 5 4 | 0 28 | 0 26 | 4 1 | 5 8 |
| IV | 15 | Mean | 3 496 | 3 257 | 138 3 | 136 5 | 3 95 | 3 [illegible]3 | 106 5 | 99 4 |
| | | S D | 0 42 | 0 41 | 2 4 | 4 3 | 0 23 | 0 30 | 2 7 | 3 2 |

Table IV *The oxygen consumption (ml/min/m²) at first (1) and second (2) examination at rest sitting and during work*

| | | | Rest | | Work | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Sitting | | 300 kpm min | | 600 kpm/min | | 900 kpm min | |
| Group | *n* | | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| I | 7 | Mean | 174 8 | 150 9 | 527 9 | 505 1 | 824 6 | 768 7 | 1218.5 | 1112.9 |
| | | S D | 16 7 | 14 5 | 45 0 | 31 0 | 48 9 | 54 6 | 94 7 | 65 8 |
| II | 9 | Mean | 150 4 | 141 1 | 502 2 | 4J7 0 | 855 8 | 790 6 | 1248 3 | 12.5 1 |
| | | S D | 9 2 | 11 5 | 46 0 | 52 3 | 64 4 | 50 3 | 162 3 | 68 9 |
| III | 7 | Mean | 158 2 | 150 0 | 539 1 | 481 9 | 837 4 | 792 4 | 1273 0 | 1141 0 |
| | | S D | 13 0 | 17 1 | 33 1 | 38 7 | 54 2 | 89 6 | 74 9 | 95 5 |
| IV | 15 | Mean | 153 3 | 148 1 | 498 3 | 495 6 | 794 7 | 795 6 | 1149 6 | 1150 9 |
| | | S D | 18 5 | 16 1 | 36 0 | 49 0 | 61 9 | 42 1 | 76 1 | 93 7 |

after therapy at rest in all body positions (Tables V–VII) in all groups The MAP at rest sitting was reduced 21 % in group II 15 % in group III and 18 % in group IV The mean values for the SAP and DAP at rest sitting were reduced to values within the limits for normotension according to WHO s criteria There was no orthostatic pressure drop before or after therapy (group IV) Before therapy the MAP in the standing position was 7 8 mm Hg higher than in the supine i after theraphy the difference was 8 8 Hg.

uring *exercise* the pressures were reduced y or almost significantly at all work levels in all three groups The relationship between $VO_2$ and MAP before and after therapy is demonstrated in Figs 2–4 There is a clear reduction of pressure in all groups

*The cardiac index (CI)*

In all groups the cardiac index in the sitting position *at rest* tended to be lower after therapy (Table VIII) In group II the difference was almost significant It is seen from Table VIII that the mean values before therapy was quite similar After therapy the reduction was 17 % in group II 10 % in group III and 5 % in group IV In the supine position (group IV) the CI after therapy

Table V *The systolic arterial pressure (mm Hg) at first (1) and second (2) examination at rest and during work*

| | | | Rest | | | | | | Work | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Supine | | Sitting | | Standing | | 300 kpm/min | | 600 kpm min | | 900 kpm min | |
| Group | *n* | | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 1 | 7 | Mean | 145 0 | 133 2 | 157 7 | 150 4 | 147 6 | 138 2 | 176 4 | 165 0 | 188 3 | 180 0 | 204 3 | 203 9 |
| | | S D | 14 1 | 8 3 | 15 7 | 22 3 | 9 5 | 11 8 | 20 7 | 21 8 | 25 6 | 23 7 | 28 8 | 6 5 |
| II | 9 | Mean | | | 182 6 | 138 2 | | | 213 4 | 168 7 | 231 4 | 187 2 | 250 7 | 215 3 |
| | | S D | | | 29 3 | 15 0 | | | 25 7 | 19 0 | 29 9 | 18 2 | 4 1 | 17 2 |
| III | 7 | Mean | | | 168 2 | 139 9 | | | 195 0 | 162 4 | 211 6 | 182.4 | 237 3 | 09 1 |
| | | S D | | | 11 5 | 11 0 | | | 17 0 | 17 6 | 18 4 | 15 4 | 22.2 | 13 7 |
| IV | 15 | Mean | 164 6 | 133 1 | 174 7 | 139 8 | 172 0 | 144 5 * | 194 4 | 163 7 * | 215 7 | 184 9 | 236 6 | 206 5 * |
| | | S D | 18 6 | 10 5 | 21 7 | 13 6 | 16 0 | 17 1 | 26 3 | 14 3 | 28 5 | 16 6 | 25 6 | 18 3 |

Table VI *The diastolic arterial pressure (mm Hg) at first (1) and second (2) examination at r st and during work*

| | | | Rest | | | | | | Work | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Supine | | Sitting | | Standing | | 300 kpm min | | 600 kpm m n | | 900 kpm min | |
| Group | *n* | | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | | 1 | |
| I | 7 | Mean | 82 0 | 75 0 | 92.9 | 87 1 | 92 8 | 88 6 | 93 0 | 85 7 | 93 8 | 8 6 | 99 3 | 95 6 |
| | | S D | 8 0 | 6 6 | 9 1 | 10 9 | 8 3 | 8 4 | 1 3 | 12 1 | 14 | 13 3 | 1 5 | 16 3 |
| II | 9 | Mean | | | 108 0 | 86 7 | | | 115 4 | 92 6 | 119 1 | 95 6 | 1 5 | 1 |
| | | S D | | | 11 1 | 11 7 | | | 11 9 | 11 1 | 15 5 | 9 | | 8 0 |
| III | 7 | Mean | | | 102.4 | 88 1 | | | 103 1 | 89 3 | 106 9 | 96 0 | 1 | 6 0 |
| | | S D | | | 7 8 | 7 1 | | | 10 4 | 13 3 | 13 7 | 13 8 | 1 4 | 10 7 |
| IV | 15 | Mean | 98 9 | 81 9 | 105 1 | 84 7 | 108 9 | 91 7 | 104 8 | 88 4 | 109 1 | 94 9 | 115 | 106 6 |
| | | S D | 7 6 | 8 1 | 10 5 | 7 4 | 8 3 | 8 8 | 9 2 | 8 5 | 12 | 8 8 | 9 5 | 10 7 |

was slightly and insignificantly reduced (0 18 l/min/m or 5° ) The changes in the CI induced by changes in body position (group IV) were not enhanced by therapy Before therapy the difference between supine and sitting CI was 0 54 l/min/m (16 % reduction) and between supine and standing CI 0 94 l/min/m (27 % reduction) After therapy the same differences were 0 40 (15 % reduction) and 0 70 l/min/m (21 reduction)

*During exercise* the CI after therapy tended to be lower than before However in the thiazide groups (III and IV) the changes were insignificant and when considered in relation to the oxygen uptake (Figs 3 and 4) the curves before and after therapy practically overlap

In the chlorthalidone group (II) however the CI after therapy was significantly reduced and the reduction is evident also when the oxygen uptake is taken into consideration (Fig )

### *The heart rate (HR) and stroke index (SI)*

Tables IX and X and Figs 2–4 demonstrate that the changes in CI are due to changes in the SI the HR being practically unaltered after therapy

### *The total peripheral resistance index (TPRI)*

Before therapy all subjects had elevated TPRI at rest (> 2700 dyn sec cm m )

It is seen in Table XI that in the sitting position *at rest* the TPRI dropped in all groups but

Table VII *The mean arterial pressure (mm Hg) at first (1) and second (2) examination at rest and during work*

| | | | Rest | | | | | | Work | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Supine | | Sitting | | Standing | | 300 kpm m n | | 600 kpm m n | | 900 kpm m n | |
| Group | *n* | | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| I | 7 | Mean | 107 4 | 95 8 | 114 7 | 111 1 | 11 0 | 108 6 | 127 4 | 116 4 | 126 4 | 121 6 | 136 4 | 135 4 |
| | | S D | 8 4 | 8 1 | 13 5 | 14 8 | 9 2 | 9 5 | 13 0 | 16 4 | 19 9 | 17 2 | 19 6 | 0 8 |
| II | 9 | Mean | | | 135 0 | 106 7 | | | 155 1 | 124 7 | 164 7 | 132 6 | 175 4 | 152 0 |
| | | S D | | | 15 0 | 12 7 | | | 18 9 | 13 6 | 22 0 | 12 3 | 14 8 | 10 4 |
| III | 7 | Mean | | | 1 8 9 | 110 0 | | | 143 4 | 118 3 | 149 6 | 130 7 | 172 1 | 147 9 |
| | | S D | | | 8 6 | 11 1 | | | 13 6 | 16 1 | 15 3 | 14 4 | 13 4 | 14 1 |
| IV | 15 | Mea | 124 3 | 10 7 | 131 1 | 107 2 | 13 1 | 111 5 | 143 4 | 1 0 4 | 151 9 | 127 3 | 163 2 | 146 |
| | | S D | 12 1 | 9 2 | 13 8 | 8 6 | 10 0 | 12 0 | 16 3 | 12 6 | 18 9 | 8 5 | 15 0 | |

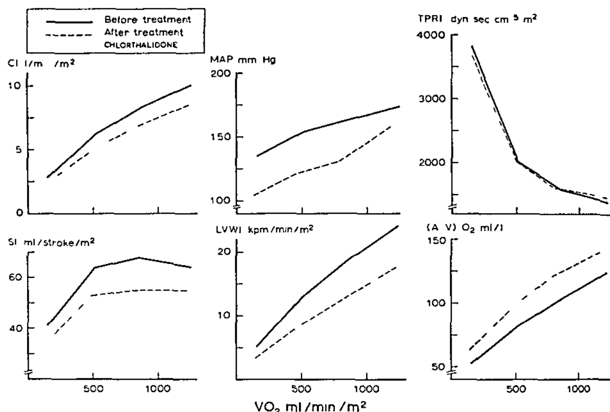



*Fig 2* Hemodynamic parameters in relation to oxygen consumption before and after therapy in the chlorthalidone group (Symbols as in Fig 1)

ıe difference between the values before and after is significant only in group IV The drop ı was only 3 % in group II 6 % in group but 14 % in group IV

In group IV the drop in TPRI was significant or almost significant in all body positions

*During muscular exercise* the TPRI was lower after therapy than before in the thiazide groups (Table XI and Figs 3 and 4) but in the chlorthalidone group the mean value was unchanged at 300 kpm/min and increased at the higher work levels (Fig 2)

Table VIII *The cardiac index (l/min/m²) at first (1) and second (2) examination at rest and during work*

| | | | Rest | | | | | | Work | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Supine | | Sitting | | Standing | | 300 kpm/min | | 600 kpm/min | | 900 kpm/min | |
| Group | *n* | | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| I | 7 | Mean | 4 31 | 3 80 | 3 39 | 2 97 | 2 87 | 2 60 | 6 39 | 5 87 | 8 45 | 8 06 | 10 38 | 10 15 |
| | | S D | 0 76 | 0 38 | 0 47 | 0 44 | 0 65 | 0 27 | 0 96 | 0 92 | 1 21 | 1 09 | 1 76 | 1 17 |
| II | 9 | Mean | | | 2 88 | 2 41 | | | 6 16 | 5 06 | 8 37 | 6 70 | 10 07 | 8 49 |
| | | S D | | | 0 36 | 0 49 | | | 0 50 | 0 76 | 0 66 | 0 99 | 0 50 | 1 23 |
| III | 7 | Mean | | | 2.94 | 2 64 | | | 5 93 | 5 29 | 7 32 | 7 32 | 9 23 | 8 91 |
| | | S D | | | 0 49 | 0 42 | | | 0 50 | 0 55 | 1 09 | 0 58 | 0 74 | 1 08 |
| IV | 15 | Mean | 3 44 | 3 [illegible]6 | 2 90 | 2 75 | 2 50 | 2 56 | 5 62 | 5 56 | 7 52 | 7 38 | 9 35 | 8 98 |
| | | S D | 0 33 | 0 34 | 0 39 | 0 [illegible]9 | 0 [illegible]6 | 0 49 | 0 99 | 0 54 | 0 82 | 0 82 | 1 20 | 1 09 |

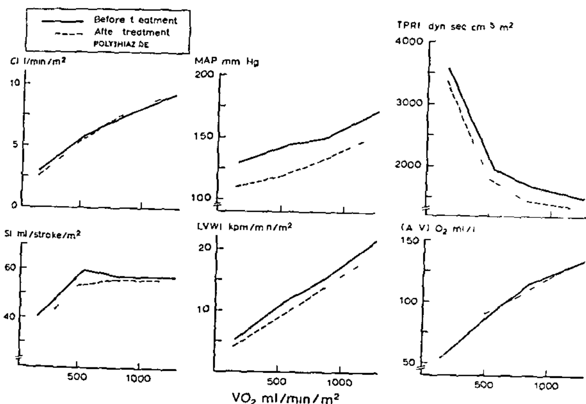



*Fig 3* Hemodynamic parameters in relation to oxygen consumption before and after therapy in the polythiazide group (Symbols as in Fig. 1)

*The left ventricular work index and the arterio-venous difference*

The changes in these calculated data are shown in Figs 2–4 The LVWI was reduced in all three groups both at rest and during exercise The arteriovenous difference showed no or minor changes in groups III and IV but was increased in group II both at rest and during work

*Individual changes in pressure and resistance*

Table XII demonstrates the individual changes in pressure and resistance It is seen that in all groups most patients obtained at least 10% reduction in MAP at rest and during exercise but a similar reduction in *TPRI* was found less frequently In the chlorthalidone group none of the patients obtained 10% reduction in TPRI at rest or during two work loads in contrast to the hydrochlorothiazide group where most patients did so

## DISCUSSION AND CONCLUSION

### A *Untreated Group*

The reduction in VO at rest in this group could be due to less apprehension at the second study and so could the lower CI The other data showed only minor changes and at the highest work level the mean values at the first and second study were nearly identical

### B *Treated Groups*

The changes in blood pressure and serum electrolytes agree well with previous studies (3 16) The reduction in plasma volume seen 8–12 months after continuous treatment contrasts with earlier investigations (3 32) but agrees well with more recent studies (2 10) A reduction of 0 239 l or 7% is very close to the values reported by Hansen (10) after 75 mg daily for 3 months

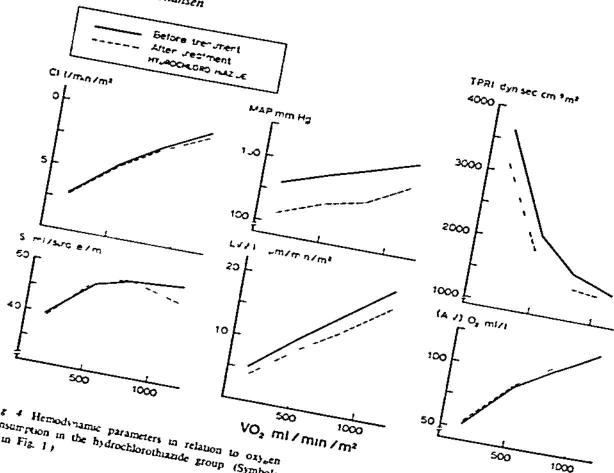



*Fig 4* Hemodynamic parameters in relation to oxygen consumption in the hydrochlorothiazide group (Symbols as in Fig. 1)

In all groups the CI was lower at the second examination This contrasts with the well known by Conway and Lauwers (3) who reported ...ase in supine CI from 3 7 to 4 1 1 min m² ...0 5 g chlorothiazide daily during several ...s (mean 5 4 months) However in the thiazide groups the reduction in flow was small and insignificant and did not persist during exercise In accordance with Conway and Lauwers a decrease in TPRI was found in the thiazide groups

It is believed that the hypotensive mode of action of chlorthalidone and thiazides is similar (7) This was not found in the present study

Table IX *The heart rate (beats min) at first (1) and second (2) examination at rest and during work*

| Group | n | | Rest: Supine 1 | Supine 2 | Sitting 1 | Sitting 2 | Standing 1 | Standing 2 | Work 300 kpm min 1 | 300 kpm min 2 | 600 kpm min 1 | 600 kpm min 2 | 900 kpm min 1 | 900 kpm min 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 7 | Mean | [illegible] | | 81.0 | 5.4 | 99.6 | 66.8 | 105.3 | 101.4 | 134.6 | 130.1 | 161.[illegible] | 157.1 |
| | | S D | 6[illegible] | 11.1 | 10.0 | 11.7 | 21.6 | 15.8 | 10.0 | 11.7 | 18.4 | 18.1 | [illegible]9 | 1.3 |
| II | 9 | Mean | | | 0.4 | 69.6 | | | 97.8 | 95.8 | 1[illegible]4.7 | 1[illegible]1.1 | 158.7 | 155.6 |
| | | S D | | | 12.1 | 6 | | | 13.0 | 1[illegible]1 | 15.6 | 14.7 | 16.1 | 0.3 |
| III | 7 | Mean | | | [illegible]0 | 4.5 | | | 103.0 | 100.6 | 130.7 | 133.4 | 166.6 | 16[illegible].8 |
| | | S D | | | 1 4 | 9.5 | | | 17.0 | 14.1 | 19.[illegible] | 18.1 | 18.7 | 2[illegible].7 |
| IV | 15 | Mean | 69.5 | [illegible]8.5 | 73.9 | [illegible]5 | 81.9 | 8[illegible].3 | 104.4 | 100.6 | 132.5 | 129.6 | 161.6 | 1[illegible]0.8 |
| | | S D | 7.1 | 8.0 | 8 | 8.4 | 11 | 10.1 | 10.[illegible] | 10.3 | 13.3 | 15.2 | 15.1 | 1[illegible]0 |

Table X *The stroke index (ml/stroke/m²) at first (1) and second (2) examination at rest and during work*

| | | | Rest | | | | | | Work | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Supine | | Sitting | | Standing | | 300 kpm min | | 600 kpm min | | 900 kpm min | |
| Group | *n* | | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| I | 7 | Mean | 56 7 | 53 4 | 42.2 | 40 3 | 30 3 | 30 8 | 60 7 | 58 3 | 63 3 | 62 6 | 65 3 | 65 4 |
| | | S D | 9 7 | 8 1 | 7 0 | 9 6 | 10 5 | 7 1 | 8 5 | 11 2 | 10 6 | 10 1 | 13 4 | 10 2 |
| II | 9 | Mean | | | 41 4 | 34 8 | | | 63 8 | 53 2 | 67 9 | 55 4 | 64 | 54 9* |
| | | S D | | | 4 8 | 6 8 | | | 9 1 | 3 2 | 9 3 | 5 5 | 8 4 | 7 1 |
| III | 7 | Mean | | | 40 5 | 35 4 | | | 58 6 | 53 0 | 56 4 | 55 7 | 56 1 | 55 2 |
| | | S D | | | 4 3 | 3 0 | | | 7 2 | 5 5 | 7 8 | 8 3 | 6 5 | 6 5 |
| IV | 15 | Mean | 49 8 | 48 0 | 39 8 | 38 0 | 31 1 | 32 4 | 54 1 | 55 8 | 57 3 | 57 4 | 58 7 | 52 8 |
| | | S D | 5 9 | 6 4 | 7 2 | 6 4 | 6 2 | 7 6 | 9 6 | 8 1 | 9 4 | 7 5 | 9 8 | 7 8 |

Table XI *The total peripheral resistance index (dyn sec cm⁻⁵m²) at first (1) and second (2) examination at rest and during work*

| | | | Rest | | | | | | Work | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Supine | | Sitting | | Standing | | 300 kpm min | | 600 kpm min | | 900 kpm min | |
| Group | *n* | | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| I | 7 | Mean | 2040 | 036 | 2777 | 3079 | 3235 | 3384 | 1593 | 1632 | 1216 | 1 9 | 1081 | 1082 |
| | | S D | 410 | 316 | 649 | 774 | 669 | 575 | 388 | 403 | 238 | 258 | 253 | 222 |
| II | 9 | Mean | | | 3809 | 3688 | | | 2036 | 2017 | 1582 | 1619 | 1393 | 1466 |
| | | S D | | | 724 | 991 | | | 367 | 384 | 240 | 3 5 | 107 | 277 |
| III | 7 | Mean | | | 3580 | 3367 | | | 1952 | 1802 | 1674 | 1446 | 1496 | 1348 |
| | | S D | | | 641 | 393 | | | 297 | 284 | 381 | 259 | 188 | 237 |
| IV | 15 | Mean | 2870 | 2543 | 3657 | 3145 | 4279 | 3590 | 2133 | 1740 | 1638 | 1396 | 1410 | 13 5 |
| | | S D | 434 | 307 | 487 | 355 | 574 | 751 | 460 | 177 | 295 | 179 | 212 | 195 |

where the thiazides mainly reduced vascular resistance and chlorthalidone mainly reduced cardiac output The cause of this difference is not known It cannot be explained on the basis of a different effect on serum potassium since this was reduced similarly in all treated groups

The study demonstrates that in the majority of patients with moderate essential hypertension and no or slight complications it is possible by long acting or short acting thiazide preparations to reduce the elevated total peripheral resistance both at rest and during muscular exercise in other words to *normalize* the systemic circulation This could support the idea of treating even subjects with mild and moderate hypertension

In this study chlorthalidone had the most pro-

Table XII *Individual changes in mean arterial pressure (MAP) and total peripheral resistance (TPRI) after therapy (in untreated group at second examination in relation to the first)*

| | MAP Reduced > 10 | | TPRI Reduc d > 10 | |
|---|---|---|---|---|
| Group | R st sitt ng | Rest d 2 work loads | Rest s tt ng | Rest and 2 work loads |
| Untreated (7) | 0 | 0 | 1 | 0 |
| Chlorthal done (9) | 8 | 6 | 2 | 0 |
| Polythiazide (7) | 5 | 4 | 3 | |
| Hydrochlorothiazide (15) | 13 | 9 | 10 | |

nounced antihypertensive effect but th. pressure reduction was mainly due to reduction in cardiac output and not in vascular resistance From a theoretical point of view this is not the ideal way to reduce the pressure but whether this is of clinical importance cannot be answered by the present study Nor does the study give any ex planation of the possible cause of the difference in mode of action for the two types of drugs

## REFERENCES

1 Aleksandrow D Wyszmacka, W & Gajewski, J Studies on the mechanism of hypotensive action of chlorothiazide New Engl. J Med. 260 51 1959

Baczko A., Dabrowska, B., Poplawska, W & Wocial, B Circulating blood volume in long term hydrochlorothiazide treatment Pol. Arch. Med. wewnęt. 39 403 1967

3 Conway J & Lauwers, P Hemodynamic and hypotensive effects of long term therapy with chlorothiazide Circulation 1 21 1960

4 Crosley A P Cullen, R C White D Freeman, J F., Castillo C A & Rowe C C Studies of the mechanism of action of chlorothiazide in cardiac and renal disorders J Lab clin. Med 55 182 1960

5 Dustan, H Cumming, C R Corcoran, A C & Page I H A mechanism of chlorothiazide-enhanced effectiveness of antihypertensive ganglioplegic drugs. Circulation 19 60 1959

6 Frohlich, E D Schnaper H W., Wilson, I M & Freis, E D Hemodynamic alterations in hypertensive patients due to chlorothiazide New Engl J Med. 26 1 61 1960

Goodman. L S & Gilman, A The pharmacological basis of therapeutics Macmillan New York 1965

Greene M A Boltax A J & Scherr E S Acute effects of intravenous chlorothiazide upon cardiovascular hemodynamics Amer Heart J 6. 659 1961

9 Greene M A Boltax A J N M & Rogow E The influence of chlorothiazide upon the cardiovascular response to a vasoconstrictor drug phenylephrine. Amer J med Sc 6 575 1963

10. Hansen, J Hydrochlorothiazide in the treatment of hypertension. Acta med scand 183 317 1968

11 Lund Johansen, P Hemodynamics in early essential hypertension. Acta med scand Suppl 482 1967

12 Rowe G G., Castillo C A Crosley A P Maxwell, G M & Crumpton C W Acute systemic and coronary hemodynamic effects of chlorothiazide in subjects with systemic arterial hypertension Amer J Cardiol. 10 183 1962

13 Varnauskas, E., Cramér G Malmcrona, R. & Werkö L. Effect of chlorothiazide on blood pressure and blood flow at rest and on exercise in patients with arterial hypertension. Clin. Sci. 20 406 1961

14 Varnauskas, E. & Schröder G Saluretika som antihypertensiomedel. Nord. Med. 67 710 196.

15 Villarreal, H., Exaire, J E., Revollo A. & Soni, J Effects of chlorothiazide on systemic hemodynamics in essential hypertension. Circulation 26 405 1962

16. Winer B The antihypertensive actions of benzothiadiazides. Circulation 23 211 1961

17 Wilson, I M. & Freis, E. D Relationship between plasma and extracellular fluid volume depletion and the antihypertensive effect of chlorothiazide. Circulation 20 1028 1959

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# RENAL VASCULAR CHANGES IN ANKYLOSING SPONDYLITIS

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*Abstract* Kidney biopsies revealed vascular changes in eleven out of fourteen patients with ankylosing spondylitis. Three had amyloidosis, six had severe renal vascular fibrinoid changes and two had moderate lesions. The fibrinoid vascular changes resembled those seen in other autoimmune diseases. Skin biopsies were performed in eight of the patients. No changes were noted.

In ankylosing spondylitis amyloidosis and lesions of the eye heart and aorta with its main branches are the extra articular manifestations most frequently referred to (8) Little interest has been focused on vascular changes other than those arteritides occurring in the skeletal regions affected (5 6) No reports have hitherto appeared on renal vascular changes in ankylosing spondylitis

We here report the results of renal biopsy studies in fourteen patients with ankylosing spondylitis Special attention was paid to the occurrence of renal vascular changes In eight patients a skin biopsy was studied

## MATERIAL AND METHODS

The series comprises fourteen patients with ankylosing spondylitis. The main clinical and laboratory findings appear in Table I

The diagnosis was based on the typical clinical and radiological picture including early onset, stiffness and pain in the spine sacroiliac involvement and ossification of the paraspinal ligaments. The diagnosis was further supported by absence of the rheumatoid factor

Renal function was normal in all cases. Two patients had proteinuria, and mild leukocyturia appeared transiently in six Neither pyelonephritis nor chronic urinary tract infection were present in the patients of this study However in three the history was suggestive of prostatovesiculitis. Patients for renal biopsy were selected on a volunteer basis.

Percutaneous renal biopsy was performed in all fourteen patients, using the Vim-Silverman-Boecker needle In eight patients a skin biopsy was taken from the forearm, using a punch 5 mm in diameter All biopsies were done under local anaesthesia (1 % lidocaine)

The biopsy specimens were fixed in 10 % neutral formal dehydrated embedded in paraffin wax sectioned at 3–5 $\mu$ and stained by the following methods haematoxylin-eosin, haematoxylin van Gieson, periodic acid Schiff methenamine silver Gomori s reticulin stain, and thioflavine T methyl violet and Congo red for amyloid.

## RESULTS

In 11 of the 14 cases vascular changes were noted in the kidney biopsies In some of the cases these changes were accompanied with tubular atrophy and interstitial changes The positive findings are indicated in Table I

In the skin biopsies (8 patients) no vascular changes were seen (see Table I)

The vascular changes consisted of swelling or deposits of homogeneous material on the inner wall of the vessels which stained strongly purple with the periodic acid Schiff (PAS) stain was faintly eosinophilic and gave a yellow colour with the van Gieson stain and a brownish grey with Gomori s reticulin stain They were not argyrophilic after staining with methenamine silver With thioflavine T methyl violet and Congo red the did not show the staining characteristics of a loid The swellings or deposits were lo tween the intima and the media an varying parts of the muscular layer were limited outwards by the o

Table 1 *Main clinical and laboratory findings*

| Pat | Age (y) | Sex | Clinical manifestations | Duration of disease (y) | Drugs prescribed | Latex test | Waaler-Rose | Blood pressure (mm Hg) | Urine | Creatinine clearance (ml/min) |
|---|---|---|---|---|---|---|---|---|---|---|
| A T | 35 | ♂ | Prostato-vesiculitis coxitis spondylitis | 16 | Indomethasine | — | — | 1·0 90 | — | 96 |
| E V | 30 | ♂ | Tonsillitis spondylitis | 9 | Phenylbutazone | — | — | 125/80 | Pyuria | 116 |
| S R | 33 | | Spondylitis | 12 | — | — | — | 100 65 | — | 1 6 |
| P H | 32 | ♂ | Spondylitis | 9 | Phenylbutazone | — | — | 1·0/70 | Pyuria | 1 4 |
| M T | 37 | | Spondylitis | 10 | Phenylbutazone | — | — | 135 80 | — | 160 |
| E J | 30 | ♂ | Tonsillitis coxitis spondylitis | 19 | Phenylbutazone | — | — | 1·0/70 | — | 1 0 |
| M J | 34 | ♂ | *Prostato-vesiculitis peripheral arthritis, spondylitis* | 5 | *Phenylbutazone indomethasine* | — | — | *130 85* | — | *179* |
| A O | 4 | | Psoriasis spondylitis | 10 | Phenylbutazone indomethasine | — | — | 125 85 | Pyuria | 160 |
| E T | 31 | | Tonsillitis spondylitis | 8 | Phenylbutazone | — | — | 125 90 | — | 133 |
| J R | 38 | | Coxitis spondylitis | 18 | Corticosteroids phenylbutazone indomethasine | — | — | 1·0/70 | Pyuria | 141 |
| F S | 8 | | Iritis peripheral arthritis spondylitis | 10 | Corticosteroids | — | — | 135/85 | Pyuria | |
| M R | 5 | ♂ | Coxitis spondylitis, colitis | 9 | Phenylbutazone | — | — | 1 0/70 | Proteinuria | 93 |
| M W | 34 | ♂ | Psoriasis spondylitis | 14 | Phenylbutazone | — | — | 130 80 | Proteinuria | 89 |
| J S | 36 | | Prostato-vesiculitis spondylitis | ·0 | Phenylbutazone | — | — | 135 95 | Pyuria | 72 |

(—) not performed





*Fig 1* (*a*) Renal arteriole with segmental deposit staining strongly purple with periodic acid Schiff (patient E V) PAS stain, ×670 (*b*) Renal arteriole from the same biopsy The deposit is silver negative Methenamine silver 6 0

| Kidney biopsy | | | | |
|---|---|---|---|---|
| Vascular changes | Tubular atrophy | Interstitial changes | Amyloid | Skin biopsy |
| ++ | − | − | − | − |
| ++ | + | − | − | (−) |
| ++ | − | − | − | (−) |
| ++ | + | + | − | − |
| ++ | + | + | − | (−) |
| ++ | − | | − | (−) |
| + | − | − | − | − |
| + | + | − | − | − |
| − | − | − | − | (−) |
| − | − | − | − | − |
| − | − | − | − | (−) |
| ++ | + | + | − | − |
| ++ | + | + | − | |
| ++ | + | + | − | − |

brane These changes were observed in eight of the eleven cases They were seen in arterioles and arteries Both segmental and circumferential localization occurred (Figs 1–3)

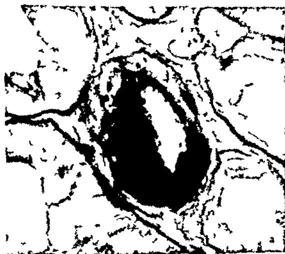
In three patients amyloidosis was diagnosed One of them (J S) revealed some notable characteristics The deposits in the renal arteries in this case stained as amyloid with thioflavine T and Congo red but showed faint metachromasia with methyl violet They differed from amyloid in giving strong purple staining with PAS and a brownish grey colour with Comori s reticulin stain Table II shows the staining characteristics of this case compared with the other two cases of amyloidosis and the rest of the cases with vascular changes

## DISCUSSION

Renal vascular changes were seen in eleven of the fourteen kidney biopsies studied In nine patients the vascular lesions were severe Three of these nine patients had amyloidosis while in the other six the vascular changes consisted of PAS-positive deposits which are best regarded as fibrinoid changes (14) Another two patients showed this change in moderate degree Only three patients could be considered normal

The frequency of renal vascular disease in this series is thus imposing A noteworthy fact is that most of the patients were young the mean age being only 34 years Another point of interest is that all the patients had normal blood pressures

The finding of a high frequency of renal vascular changes in this series of young normotensive



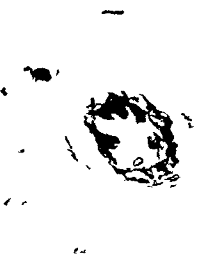


Fig 2 (a) Renal arteriol with de n segmental deposit staining strongly purple with periodic acid Schiff (patient M T) PAS stain ×670 (b) Renal arteriole from the same biopsy Note that the deposit ferential nd silver negative Meth

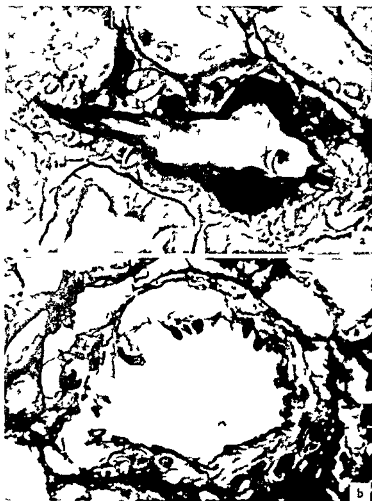


*Fig 3* (*a*) Renal artery with segmental deposits staining strongly purple with periodic acid Schiff (patient S R.) PAS stain, × 540 (*b*) Renal artery from the same biopsy The deposits are silver negative Methenamine silver × 540

suggests two possibilities as regards aetiol

vascular changes might b related either to the disease per se or to its rather uniform treat ment

As possible causes of drug induced renal vascular lesions both corticosteroids (10) and phenylbutazone must be considered Corticosteroids had been used in only two cases both had normal renal arteries and arterioles Phenylbutazone has been regarded as the cause of severe necrotizing arteritis in one fatal case (12) In our series phenylbutazone had been used in all but two cases The fact that these two patients had severe fib-

Table II *Comparison of staining characteristics of renal vascular deposits in three cases of amyloidosis and eight cases with fibrinoid vascular changes (ankylosing spondylitis)*

| Pats | | Thioflavine T green fluorescence in UV light | Congo red | Methyl violet metachromasia | Gomori s reticulin stain green | PAS purple |
|---|---|---|---|---|---|---|
| M R | Amyloidosis | | | + | – | + – |
| M W | Amyloidosis | | | + | – | + – |
| J S | Amyloidosis | | | + – | + | – |
| Cases with fibrinoid changes | | | | – | + | + |

rinoid changes in the renal arteries and arterioles makes it appear unlikely that phenylbutazone was an aetiological factor

If the fibrinoid renal vascular changes observed in this series are due to the disease per se one would expect them to occur in nearly related diseases

In rheumatoid arthritis vascular lesions have been reported with varying frequency (4 9) Many investigators have found renal vascular changes (1 2 3 11 13 15 16 17) Most descriptions cover a wide spectrum of arteritic changes thought to b manifestations of the rheumatoid disease In some studies however no such specific changes have occurred (2 13 15) while arterio- and arteriolosclerosis have been present in old patients The renal vascular changes that we have seen in this material differ significantly from the arteritic changes of rheumatoid arthritis They show a greater resemblance to the non specific sclerotic changes reported but the low age of the patients suggests some other aetiology

In most diseases belonging to the group of autoimmune or connective tissue disorders vascular changes occur and the renal vasculature is often affected Fibrinoid changes in the renal arteries and arterioles are a common finding (14) Though the serological findings in ankylosing spondylitis differ from those of many autoimmune diseases this condition most probably belongs to the same group (7) and the fibrinoid vascular changes are perhaps best understood on this basis

Hypertension and diabetes mellitus and other causes of fibrinoid renal vascular changes are excluded as aetiological factors because of their absence from this series

The intermediate staining properties of the vascular deposits in one of the patients with amyloidosis are interesting They raise the question of whether the fibrinoid changes observed may in part be precursors of amyloidosis

The prognostic significance of the renal vascular changes is hard to evaluate In some of the cases the renal biopsies revealed changes apparently due to vascular alterations It is possible that the leucocyturia present in some of these cases was a reflection of these renal lesions

The normal structure of the arterioles and capillaries in all skin biopsies shows that in ankylosing spondylitis vascular changes are infrequent at that level of the vascular tree But at the level of arteries and arterioles they may b widely disseminated in other viscera besides the kidney

## REFERENCES


1 Ball J Rheumatoid arthritis and polyarteritis nodosa Ann rheum. Dis. 13 277 1954

2 Brun C Olsen T S Raaschou F & Sorensen A W S Renal biopsy in rheumatoid arthritis Nephron 2 5 1965

3 Bywaters E G L Peripheral vascular obstruction in rheumatoid arthritis and its relationship to other vascular lesions Ann. rheum Dis 16 84 1957

4 Cruickshank, B The arteritis of rheumatoid arthritis. Ann rheum. Dis. 13 136 1954

5 — Pathology of ankylosing spondylitis Ann rheum Dis. 10 211 1960

6 Engfeldt B Romanus, R. & Yden S Histological studies of pelvo spondylitis ossificans (ankylosing spondylitis) correlated with clinical and radiological findings Ann rheum Dis 12 219 1954

7 Grimble A Anti prostate antibodies in arthritis Brit med. J 2 63 1963

8 Hart, F D Ankylosing spondylitis Lancet 1340 1968

9 — Rheumatoid arthritis extra articular manifestations Brit med J 3 1311 1969

10 Kemper J W Baggenstoss A H & Slocumb C H The relationship of therapy with cortisone to the incidence of vascular lesions in rheumatoid arthritis. Ann intern. Med 46 831 1957

11 Mahallawy M N El & Sabour M S Renal lesions in rheumatoid arthritis Lancet 85 1959

12 Nathan D A Meltus M L Capland L & Lev M Death following phenylbutazone (Butazolidin) therapy report of a case Ann intern Med 39 1096 1953

13 Pasternak, A Wegelius, O & Makisara, P Renal biopsy in rheumatoid arthritis Acta med scand 182 591 1967

14 Pirani C L & Manaligod, J R The kidneys in collagen diseases In The Kidney (eds F K Mostofi & D E Smith) pp 147–03 Williams & Wilkins Co Baltimore 1966.

15 Pollak, V E Pirani, C L Steck, I E & Kark R M The kidney in rheumatoid arthritis studies by renal biopsy Arthr and Rheum 5 1 1962

16 Sinclair R. J G & Cruickshank B A clinical and pathological study of sixteen cases of rheumatoid arthritis with extensive visceral involvement ("Rheumatoid Disease") Quart. J Med 25 313 1956

17 Sokoloff L & Bunim, J J Vascular lesions in rheumatoid arthritis. J chron Dis 5 668 1957

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# FAT MOBILIZING LIPOLYSIS AND LEVELS OF CYCLIC AMP IN HUMAN AND DOG ADIPOSE TISSUE

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*Abst act* Intact omental human adipose tissue slices as well as isolated adipocytes from the same source re pond in vitro to noradrenaline plus caffeine with increased lipolysis and tissue levels of cyclic AMP Dog omental adipose tissue also responds in vitro with increased levels of cyclic AMP and stimulation of lipolysis when exposed to caffeine and/or noradrenaline By contrast ACTH alone or in combination with caffeine is without effect on cyclic AMP levels or lipolysis in both species. The results suggest that cyclic AMP may be a regulator of l polysis in human fat and they lend further support to the hypothesis that l polytic hormones act through in creased cyclic AMP levels

The physiologic and clinical importance of the mobilization of free fatty acids (FFA) from adi pose tissue has recently been reviewed (5 7) One of the key processes regulating mobilization of FFA is lipolysis of the stored triglycerides in adi pose tissue It has been shown in rat adipose tis sue that one important regulator of fat mobilizing lipolysis is the intracellular levels of adenosine 3 5 monophosphate (cyclic AMP) Cyclic AMP is synthesized from ATP by a membrane enzyme system adenyl cyclase which is activated by lipo lytic hormones for example catecholamines and ACTH (2) and is destroyed by a phosphodiesterase which is inhibited by the methyl xanthines e g theophylline or caffeine (4) Increased tissue levels of cyclic AMP by either of these mechanisms re sulted in increased lipolysis in rat fat (3)

Human fat when studied in vitro reacts in some but not all respects in a way which suggests that cyclic AMP may be a regulator of lipolysis also in this tissue Thus catecholamines and theophyl line stimulate lipolysis in human fat (8) as in rat fat On the other hand ACTH does not increase lipolysis in human adipose tissue (6) Against this background this study was undertaken to establish whether there existed in human adipose tissue a correlation between fat mobilizing lipolysis and cyclic AMP a question as yet not studied Since we had seen that dog fat behaved similarly in vitro from the lipolytic point of view to human fat with regard to the response to catecholamines and ACTH (6) studies were also made in this sp cies

## MATERIAL AND METHODS

Omental adipose tissue was obtained at surgery for non acute non icteric gall bladder disease The p tients were fast ng over night and premedicated w th atrop ne and Oxicon® (Aco Stockholm, Sweden) Anesthesia was in duced w th Narkotal® (Astra, Sodertalje Sweden) and continued w th Halothane® (ICI England) $N_2O$ and oxygen. Respiration was controlled, the trachea being n tubated as a routine Sal ne was g en a a slow intra venous infusion

The tissue obtained w thin 30 min after induct on of nesthesia, was placed in incubation medium, mmediately brought to the labo atory and processed as desc bed in deta l pre ously (8) After transfer of the adipose t ue p ces to incubation fla ks th re was a p eincubation period of 20 m n Appropri te dd t ons we e made into the flasks, which were then ncubated for 10 m n The fla ks conta ned about 1 g of tiss e n 5 ml of Kr bs-R nger bicarbonate buffer pH 7 4 2% human alb min (Kabi, Stockholm, Sweden) and 0 1% glucose N lue (Nor Exadrin co c ® Ast a, Sodertal e f ine (Aco Stockholm, Sw den) ACT Ph rmac a, Uppsala, Sweden) and 0 9

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Table I. *Glycerol release (μmol/g/h) from and levels of cyclic AMP (pmol/g) in human omental adipose tissue slices incubated in vitro*

Mean value ± S.E.M. obtained from in most instances 4 incubation flasks, in a few from 3 flasks. Noradrenaline 10 μg/ml, caffeine 10⁻³ M, ACTH 0.1 U/ml

| | Addition to the medium | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NaCl | | Noradrenaline | | Caffeine | | Noradrenaline + caffeine | | ACTH | | ACTH + caffeine | |
| Pat. | Glycerol | Cyclic AMP | Glycerol | Cyclic AMP | Glycerol | Cyclic AMP | Glycerol | Cyclic AMP | Glycerol | Cyclic AMP | Glycerol | Cyclic AMP |
| | 0.14 ± 0.0_ | _90 ± 25 | | | | | 1.82 ± 0.02 | 510 ± 30 | | | | |
| | 0.12 ± 0.06 | _80 ± 36 | | | | | 1.90 ± 0.14 | 1560 ± 1_0 | | | | |
| b | 0.__ ± 0.04 | 93 ± _ | | | | | 1.11 ± 0.05 | 280 ± _0 | | | | |
| | 0._0 ± 0.07 | 110 ± 10 | | | | | 1.11 ± 0.06 | 320 ± _0 | | | | |
| | 0.1? ± 0.03 | 100 ± _0 | 0.84 ± 0.14 | 190 ± 13 | 0.48 ± 0.10 | 130 ± 3 | 2.00 ± 0.14 | 190 ± 10 | 0.22 ± 0.07 | 110 ± 60 | 0.29 ± 0.11 | 100 _7 |
| | 0.27 ± 0.08 | _00 ± 30 | 0.95 ± 0.13 | 390 ± 40 | 0.80 ± 0.19 | 2_0 ± 60 | 2.93 ± 0.25 | 490 ± 30 | 0.53 ± 0._4 | 170 ± 20 | 0.?0 ± 0.19 | _0_30 |
| | 0.31 ± 0.0 | 430 ± 80 | 0.89 ± 0.08 | 650 ± 120 | 0.23 ± 0.06 | 450 ± 40 | 2.18 ± 0.21 | 660 ± 60 | | | | |

Clamped with dry ice.
b Clamped with Wollenberger's clamps.
Homogenized in acid.

in a small volume (usually 0.1 ml) at the beginning of the 10 min incubation period. Glycerol release into the medium was determined as described before (6). At the end of the incubation the tissues were fixed either by clamping between aluminum blocks chilled in liquid nitrogen or by homogenization in acid and cyclic AMP was determined as described (_, 3).

Adipose tissue cells were prepared from about 10 g of omental adipose tissue by incubation of about _ g of tissue cut into small pieces, in 6 ml of Krebs-Ringer [illegible]rbonate bu[illegible] containing 3[illegible] human albumin, 0.1% [illegible] and 1 [illegible]g of collagenase (lot no. C-0130 [illegible] Chemical [illegible] St. Louis, Missouri, USA) for one [illegible] at 37 [illegible] w[illegible] [illegible] shaking in plastic Packard [illegible] The su[illegible]e [illegible] then poured through a nylon stocking into a plastic tube and kept at 37 C. The fat droplets which immediately rose to the surface were suctioned off, and after 5–10 min standing the cells had floated to the surface and the infranatant was aspirated. This washing procedure was repeated 3–4 times with 37 C buffer albumin solution. The resulting cell preparations were combined and buffer albumin added to give a final amount of 1 g of adipose tissue (original weight, the recovery was in general 50%) per ml of solution. Two ml of this cell suspension was added to incubation vessels containing 3.5 ml of the albumin-buffer solution. The vessels were preincubated for _0 min at 37 C. 0.5 ml taken off for glycerol analysis, the appropriate additions made in 0.1 or 0.05 ml, and the vessels incubated for 10 min. Then 0.5 ml was taken off for glycerol estimation and 3 ml pipetted off into 20 ml of 0.1 N HCl for determination of cyclic AMP (3). Omental adipose tissue was obtained from two mongrel dogs, which had been fasted for 24 hours. The tissues were taken during pentobarbital (Nembutal®, Abbott Lab., N. Chicago, Ill., USA) anesthesia and treated exactly as described above for human tissue.

Table II. *Glycer[illegible] lease (μmol/incubation flask/h) from and levels of [illegible] AMP (pm[illegible] ncubation flask) in adipocytes isola[illegible] from hum[illegible] mental adipose tissue incubated in vitr[illegible]*

Mean ± S.E.M. from 3 [illegible] [illegible] mean and individual values when onl[illegible] [illegible] were incubated due to lack of material. Noradrenal[illegible] [illegible] g/m[illegible] caffeine 10⁻ M

| | Addition to the medium | | | |
|---|---|---|---|---|
| | NaCl | | Noradrenaline + caffeine | |
| Pat. | Glycerol | Cyclic AMP | Glycerol | Cyclic AMP |
| 1 | 0.38 (.31–.45) | 90 ± 10 | 9_ ± 0._1 | _20 ± 10 |
| 2 | 0.54 (._4–.83) | None detectable | 1 (.04–4.79) | 2_0 (_60–_80) |

## RESULTS

Seven experiments were performed with whole intact adipose tissue from six patients. In all noradrenaline + caffeine in the medium significantly increased the glycerol release (Table I) on the average from 0.20 to 1.86 μmol/g/h. At the same time the level of cyclic AMP increased in all experiments, and the increase was statistically significant in six of the studies. The overall means for

Table III *Glycerol release (μmol/g/h) from and levels of cyclic AMP (pmol/g) in dog omental adipose tissue slices incubated in vitro*

Mean ±S E M from 4 incubation flasks Noradrenaline 10 μg/ml caffeine $10^{-3}$ M ACTH 0 1 IU/ml

| Addition to the medium | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NaCl | | Noradrenaline | | Caffeine | | Noradrenaline + caffeine | | ACTH | | ACTH + caffeine | |
| Glycerol | Cyclic AMP | Glycerol | Cyclic AMP | Glycerol | Cyclic AMP | Glycerol | Cyclic AMP | Glycerol | Cyclic AMP | Glycerol | Cyclic AMP |
| 0.70±0.13 | 70±6 | 3.24±0.35 | 210±30 | 0.66±0.10 | 90±20 | 3.80±0.24 | 250±[illegible]0 | 0.43±0.08 | 70±8 | 0.68±0.12 | 100±[illegible] |
| 0.75±0.11 | 73±6 | 1.91±0.26 | 400±40 | 1.40±0.14 | 90±10 | 1.85±0.14 | 540±50 | 1.21±0.27 | 90±10 | 0.93±0.05 | 90±10 |

cyclic AMP levels under basal conditions and after addition of noradrenaline + caffeine were 210 and 570 pmol/g respectively In three of the experiments with whole adipose tissue the effects of noradrenaline or caffeine alone were measured Noradrenaline stimulated lipolysis and increased tissue levels of cyclic AMP in all instances while caffeine alone only raised glycerol output in two of these three experiments but to a lesser degree than noradrenaline and in none was cyclic AMP significantly increased by caffeine ACTH alone or in combination with caffeine did not change significantly either the glycerol release from or the cyclic AMP levels in adipose tissue

Noradrenaline + caffeine had the same effect on human adipose tissue cells as on intact tissue (Table II) with measurable increases of both lipolytic rates and tissue content of cyclic AMP

Table III shows the results obtained in the dog studies Noradrenaline alone and noradrenaline + caffeine resulted in significant increases in the tissue levels of cyclic AMP as well as in the lipolytic rates Neither ACTH alone nor ACTH in combination with caffeine changed the glycerol production or cyclic AMP levels

## DISCUSSION

For the first time it has been shown that stimulation in vitro of lipolysis in *human* fat either in tact adipose tissue slices or isolated adipocytes is accompanied by raised tissue levels of cyclic AMP This suggests that cyclic AMP may play an important role in regulation of lipolysis also in human fat Consistent with this view is the finding that ACTH which stimulates lipolysis in rat but not in human fat (6) does not raise the adipose tissue levels of cyclic AMP Another finding lending further support to this view of a central role of cyclic AMP in regulating lipolysis in human fat is that the dibutyryl derivative of cyclic AMP at a concentration of $10^{-3}$ M stimulated glycerol release about fourfold in both rat and human fat (6)

There was a striking similarity between the response of dog and human fat to catecholamines and ACTH In contrast to rats these two species do not respond to ACTH with either increased fat mobilizing lipolysis or raised cyclic AMP levels in omental fat The parallelism between changes in cyclic AMP levels and lipolytic rates lends further support to a causal relationship between the two Thus the specificity between hormones and adenyl cyclase systems has been extended through species variations of a single tissue as well as different tissues (9)

Although fat cells from rats and humans appear to have similar general properties with regard to effects of catecholamines and caffeine on cyclic AMP and fat mobilizing lipolysis it appears that there are differences in the quantitative responses of the two species in this regard However the significance of this is not clear since other factors have not been controlled For example adipose tissue from older rats is much less responsive to catecholamine and caffeine stimulation in terms of cyclic AMP response than that from younger rats (1)

## ACKNOWLEDGEMENTS

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## REFERENCES

1 Butcher R W & Baird C L Unpub ished observa tions.

2 Butcher R. W., Baird C. E & Sutherland, E. W The effects of lipolytic and anti lipolytic substances on *adenosine 3,5 monophosphate* levels in isolated fat cells. J Biol Chem. 243 1705 1968

3 Butcher R W Ho R S Meng, H C & Sutherland, E W Adenosine 3,5 monophosphate in biological materials. II The measurement of adenosine 3 5 monophosphate in tissues and the role of the cyclic nucleotide in the lipolytic re ponse of fat to epinephrine J Biol Chem 240 4515 1965

4 Butcher R W & Sutherland E W Adenosine 3 5 pho phate *in biological materials I Purifications* and properties of cyclic 3 5 nucleotide phosphodiesterase and use of this enzyme to characterize adenosine 3 5 phosphate in human urine J Biol Chem. 237 1 44 196

Carlson L A Lipolysis of adipose tissue triglycerides Re ulation physiological and clinical significance Ex crpta Medi a International Congress Series No 161 Pr te n and polypeptide hormones Proceedings of the International Symposium Liège Belgium May 19–25 pp 143 149 1968

6 — Unpublished data

Carlson L A Boberg J & Hogstedt B Some *physiological and clinical implications of lipid mobiliza* tion from adipose tissue Handbook of physiology Adipose tissue (eds A E Renold and G F Cahill) chapter 63 pp 625–644 American Physiol Society Washing ton 1965

8 Carl cn L A Hallberg, D & Micheli H Quantitative studies on the lipclytic re ponse of human subcutaneous a l mental adipose tissue to noradrenaline and theo- r l e Acta med scand 185 465 1969

G A Butcher R W & Sutherland E W P Ann Rev Biochem 37 149 1968

Acta med scand Vol 187 pp 529–537 1970

# STUDIES IN URINARY TRACT INFECTIONS

## V Urinary Concentrating Ability

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*Abstract* Maximum urine concentrating ability (MCA) has been determined after the administration of vasopressin tannate in oil and found to be over 800 mOsm/kg in 6 of 8 normal subjects. Subnormal MCA was demonstrated in 93 of 157 bacteriuric women between the ages of 16 and 65 Impairment of MCA was positively correlated with clinical symptoms of acute pyelonephritis (fever and flank pain) and with a raised coli antibody titre In 115 of the patients MCA was determined again after five to twelve months. In 34% MCA had improved by more than 5% The assumption that this indicates acute pyelonephritis was supported by the presence of other signs of acute pyelonephritis in most of these patients in contrast to the patients who maintained a normal MCA during the observation period. It should be noted that not only Gram negative rods, but also coagulase negative staphylococci, may cause acute pyelonephritis The recurrence rate was nearly the same in patients with normal and in patients with impaired MCA before treatment, but it was significantly higher among patients in whom MCA remained low or deteriorated during the observation period than in patients whose MCA remained normal or showed improvement.

Impairment of urinary concentrating ability is often found among patients with urinary tract infection (3 5 9 10 17 22) and is considered an early sign of pyelonephritis (5 22) In chronic pyelonephritis Kleeman et al (11) and Brod et al (3) have shown concentrating ability to be relatively more impaired than would be anticipated from the reduction in glomerular filtration rate (GFR) This is probably due to the anatomical relationship between the site of infection and the concentrating apparatus Winberg (22) and others (17 19) found that the concentrating ability tended to revert to normal in acute pyelonephritis after subsidence of the acute illness

In the present study the influence of fluid deprivation on urine osmolality after administration of pitressin tannate has been investigated The concentrating ability in patients with urinary tract infection has been correlated with other signs of acute pyelonephritis and the patients have been followed to demonstrate changes in concentrating ability

## MATERIAL AND METHODS

The concentrating ability was determined in 152 non pregnant women between the ages of 16 and 65 with urinary tract infection. In no case was serum-creatinine above 13 mg l. Methods for collecting and culture of urine specimens, as well as the criteria for diagnosing bacteriuria have been described previously (13) All patients were examined after admission to hospital, where treatment was initiated. After the start of treatment the patients were followed in the outpatient clinic where mid stream specimens were collected after 1 4 and 8 weeks, and every third month thereafter During the observation period the patients were instructed to report at the clinic immediately upon recurrence of urinary tract symptoms.

The renal concentrating ability was determined as described by DeWardener (4) At 4 p m. 5 I U of Pitressin Tannate in Oil® (Parke Davis & Co) were given subcutaneously The first two urine specimens collected the next day were stored at − 18 C until a determination of the osmolality could be made This was done with an Advanced Osmometer® (accuracy within ± 1 mOsm/kg) The maximum urinary concentrating ability was determined as the highest osmolality found in the two specimens. When the test was repeated in the same patient in the outpatient clinic, the same dose of Pitressin Tannate in Oil was given at about 11 a.m and the first urine specimen collected the next morning by the patient was used for determination of osmolality

Examination for coli antibodies in the formed using the indirect haemagglutin (16) The strain isolated from the pat the antigen. In our laboratory antibody titre are < 1 80

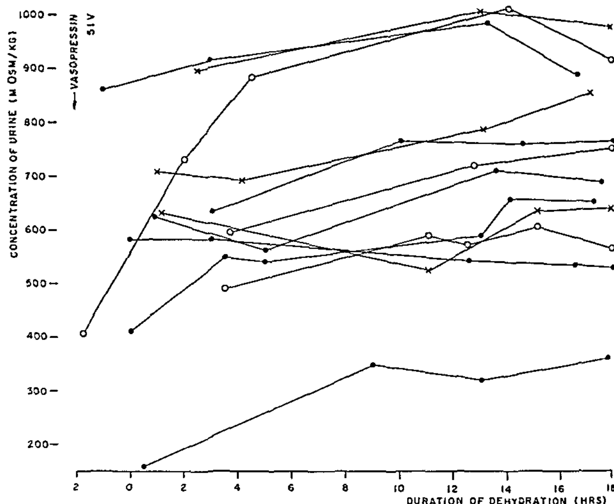



Fig 1 Osmolality of urine in 12 subjects during 18 hours dehydration Five I U of Pitressin Tannate in Oil given two hours before the test

## RESULTS

In 12 persons the maximum urinary concentrating ability was determined during continuous fluid deprivation after the administration of vasopressin Five I U of Pitressin Tannate in Oil were given at 4 p m and the patient thirsted for 18 hours starting at 6 p m During this period the osmolality of each urine specimen was determined Fig 1 shows that after over 10 hours fluid deprivation there was no further increase in urine osmolality regardless of the level of concentrating ability It is concluded that within this period all subjects had attained their maximum urinary concentrating ability

In 19 subjects the maximum concentrating ability was determined by combining vasopressin with fluid deprivation for more than 12 hours One week later the same dose of vasopressin was given at 4 p m while the fluid intake was unrestricted The osmolality of urine specimens collected the next morning was compared with the maximum osmolality achieved during the first test (Fig 2) There is no significant difference between the regression line $y = 1.06x - 3$ and $y = x$ $t$ test on the slope $t = 0.46 < t_{0.05,17} = 2.110$ $t$ test on intercept on ordinate $t = 0.03 < t_{0.05,17} = 2.1$ It is therefore concluded that maximum urinary concentrating ability (MCA) can be estimated from the osmolality of urine specimens collected 14 to 20 hours after the administration of 5 I U Pitressin Tannate in Oil regardless of fluid intake

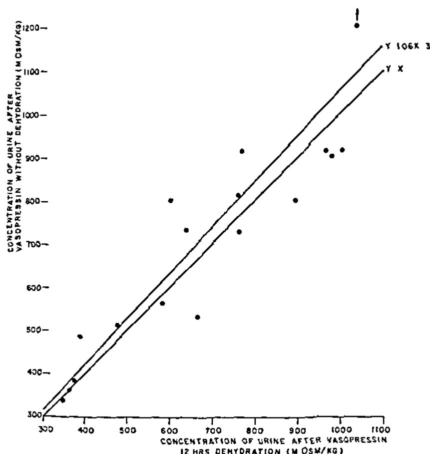



*Fig* Maximum urine osmolality (MCA) in 19 subject after 5 IU of Pitressin Tannate in Oil with (abscissa) and without (ordinate) fluid deprivation

MCA was determined in 28 persons with no history or sign of renal disease Twenty six of thes had concentrating abilities above 800 mOsm/kg this is therefore considered to be the lower limit for normal MCA (Fig 3)

In 157 women with urinary tract infection the MCA was determined and found to be below 800 mOsm/kg in 93 cases Impairment of the concentrating ability was found in 55% of 135 patients infected with Gram negative rods and in 86 of 22 patients infected with coagulase negative staphylococci (Fig 3)

In 85 patients infected with E coli the MCA was compared with other signs of acute pyelonephritis Subnormal values of MCA were found in 16 of 18 patients with fever and flank pain and in 27 of 67 patients without these symptoms ($\chi = 13\,4$ $p < 0\,001$) (Fig 4) No significant correlation was found between coli antibody titre above 1 280 and MCA below 800 mOsm/kg ($\chi = 2\,0$ $p > 0\,1$)

The relationship between the various signs of acute pyelonephritis is further illustrated in Fig 5 by a patient who developed acute pyelonephritis two weeks after treatment for urinary tract infection due to a serologically different strain of E. coli Coli antibody titre against the initial coli strain did not exceed 160

Determination of MCA was rep ated in 115 of the patients after five to twelve months Fig 6 shows MCA b fore and after this period in relation to the infecting organisms and recurrences In relation to MCA before treatment the concentrating ability was unchanged in 70 patients improved by more than 25 % in 37 cases and reduced by more than 25 in eight cases

Changes in MCA were compared with clinical symptoms of acute pyelonephritis (fever and flank pain) and with the maximum coli antibody titre in 62 patients infected with E coli (Fig 7) Eight of the 15 patients whose MCA improved by mo than 25 % showed clinical symptoms of acut

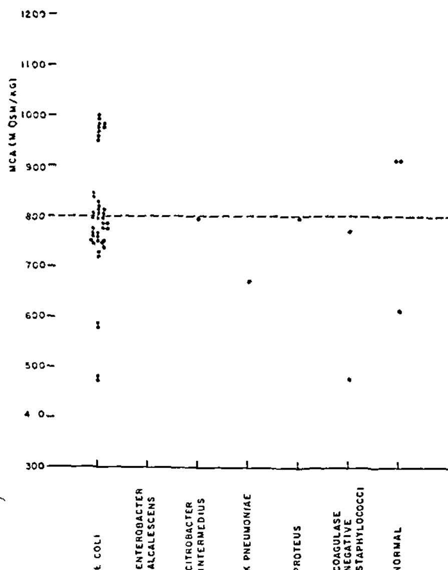



*Fig 3* Maximum urinary osmolality in 78 healthy subjects and in 157 women with urinary tract infection (grouped according to species of infecting organism)

lonephritis and six of these had a coli antibody titre above 1 280 Only one of the 25 patients with normal MCA which remained unchanged during the observation period had fever and flank pain while six of these had a coli antibody titre above 1 280 The correlation between clinical symptoms of acute pyelonephritis and improvement of MCA is statistically significant ($\chi^2$ = 12 5 $p < 0\,001$) Improvement in MCA by more than 25% during the observation period is therefore assumed to indicate acute pyelonephritis It must be noted that signs of acute pyelonephritis were found not only in patients infected with Gram negative rods but also in patients infected with coagulase negative staphylococci In seven of 14 patients infected with coagulase negative staphylococci MCA improved by more than 25 % (Fig 6)

The incidence of recrudescence and reinfection after attainment of sterile urine in 138 patients observed for more than one year has been compared with their initial MCA (Table I) Two patients had persistent bacteriuria Among 55 patients with MCA above 800 mOsm/kg 38 maintained sterile urine for more than 12 months and among 82 patients with MCA below 800 mOsm/kg, 45 maintained sterile urine during 12 months The difference between these recurrence rates is not significant ($\chi^2 = 3\,1$ 0 1 > $p > 0\,05$)

When however the material is grouped according to the changes in MCA patients with normal

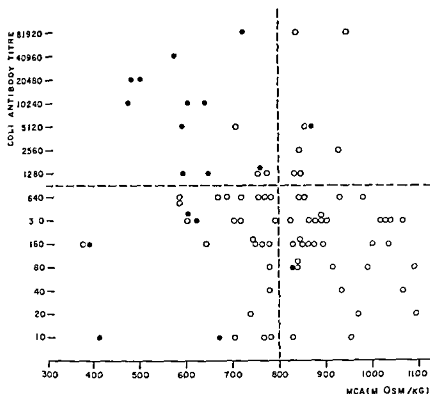



*Fig 4* Relation between MCA and coli antibody titre in 85 patients with urinary tract infection due to E coli ● indicates presence O absence of fever and flank pain

MCA or improvement in MCA maintained sterile urine significantly more frequently than did patients with MCA below 800 mOsm/kg or deterioration of MCA ($\chi^2 = 8.88$ $0.01 > p > 0.001$) It seems evident therefore that MCA is related to recurrence rate

## DISCUSSION

The clinical diagnosis of pyelonephritis in bacteriuric patients should be restricted to cases accompanied by signs of renal involvement In accordance with this acute pyelonephritis could be defined as pyelonephritis with reversible signs of renal involvement.

Due to the focal nature of the lesion in acute pyelonephritis the best method of diagnosis seems to be quantitative culture of urine obtained from the ureters (20) This procedure is not, however suitable for routine clinical work, and the diagnosis must rest upon indirect methods The most specific and useful of these are probably the observation of clinical symptoms such as fever and flank pain demonstration of reversible impairment of renal tubular function quantitative culture of urine after bladder washout as described by Fairley et al (7) and the demonstration of a rise in the titre of antibodies against the organisms isolated from the urine

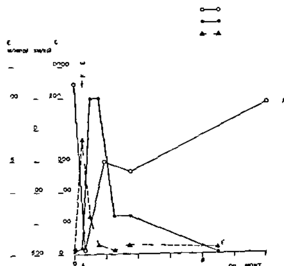
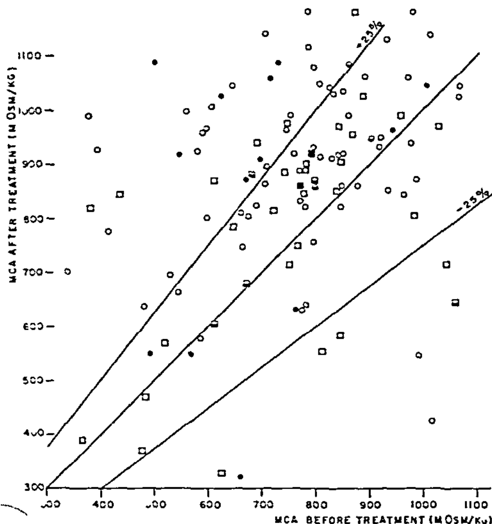


*Fig 5* MCA coli antibody titre (CAT) and ESR in a case of acute pyelonephritis.




Comparison of MCA in 115 women with urinary infection before treatment (abscissa) and after 5 months (ordinate). ○ E. coli — recurrence between two tests. □ E. coli — recurrence between the two tests. ● other Gram-negative rods — recurrence between the two tests. ▣ other Gram-negative rods, — recurrence between the two tests. ● coag.-neg. staphylococci — recurrence between the two tests. ■, coag.-neg. staphylococci — recurrence between the two tests.

Estimation of renal concentrating ability is the most convenient and widely used clinical test of renal tubular function. Due to the constancy of plasma osmolality the concentrating ability can be expressed as the maximum osmolality of the urine while specific gravity gives only an indirect and inaccurate expression of urine concentration (1, 15).

Miles et al. (15) showed in 13 normal subjects that at least 22 hours fluid deprivation was necessary to achieve 90% or more of the urine osmolality reached after 30 hours dehydration. To avoid the inconvenience of prolonged dehydration a long-acting vasopressin preparation may be used as an alternative stimulus to urine concentration. DeWardener (4) in 21 patients with impaired urine concentrating ability found good correlation between the urine osmolality after administration of Pitressin Tannate in Oil and the urine osmolality achieved after 36 to 48 hours of fluid deprivation, while normal subjects achieved somewhat higher osmolality after dehydration than with vasopressin. In children Winberg (21) found that urine osmolality after vasopressin tended to increase during up to 17 hours of fluid deprivation. In the present study no significant difference was detected between urine osmolality after vasopressin with and without fluid deprivation.

As in other studies (5, 8, 9, 17) it was found

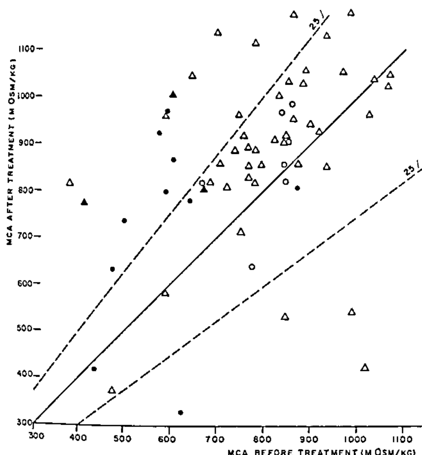



Fig 7 Comparison of changes in MCA clinical symptoms, and coli antibody titre in 67 patients infected with E coli. ● fever and flank pain present coli antibody titre ≥1 80 ○ fever and flank pain absent coli antibody titre ≥1 80 ▲ fever and flank pain present coli antibody titre < 1 80 △ fever and flank pain absent coli antibody titre < 1 80

that the lower limit of normal MCA is about 800 mOsm/kg Lewis and Alving (12) found that in normal subjects the maximum specific gravity of urine does not fall until after 65 years of age

Reduction of urinary concentrating ability is frequently reported in urinary tract infection (5 9 10 17 22) and is considered an early sign of pyelonephritis Norden and Tuttle (17) found that ten of 24 pregnant women with bacteriuria were unable to concentrate their urine above 700 mOsm/kg while all subjects in a matched group of pregnant women without bacteriuria could concentrate to above 700 mOsm/kg In a similar study Kaitz and London (10) found MCA below 700 mOsm/kg in nine of 20 pregnant women with bacteriuria while none of the controls were below 724 mOsm/kg In 22 children with non-obstructive urinary tract infection Winberg (22) found reduced concentrating ability in 17 cases and in the present study MCA below 800 mOsm/kg was found in 93 of 157 women with bacteriuria

Impairment of renal concentrating ability in advanced chronic pyelonephritis is to some extent due to a reduction in the number of functioning nephrons and the consequent increase in osmotic solute load per nephron (2 11) In chronic pyelonephritis however there is a more marked interference with the concentrating process as first pointed out by Raaschou (18) than would be anticipated solely from the reduction in GFR (3 11) This probably indicates damage to the mechanism responsible for maintenance of the hypertonicity of the medulla or in some cases to acquired nephrogenic diabetes insipidus" (6 1 )

Table I. *Number of patients with recrudescence or reinfection during a twelve months' observation period after sterile urine has been attained*

| Initial MCA | Persistent bacteriuria | Recrudescence | Reinfection | Sterile urine for 12 mo | Total |
|---|---|---|---|---|---|
| 800 mOsm/kg | 1 ( ) | 7 (13 ) | 10 (18 ) | 38 (68 ) | 56 |
| 800 mOsm/kg | 1 (1 ) | 1 (14 ) | 26 (31 ) | 45 (54 ) | 84 |

i.e. failure of the distal convoluted tubules and collecting tubules to respond to antidiuretic hormone.

A subnormal MCA in patients with bacteriuria does not establish the diagnosis of acute pyelonephritis because the concentrating ability may be impaired in consequence of previous renal disease. It was therefore of great importance when Winberg (21) demonstrated that the impairment of concentrating ability was reversible in children with acute urinary tract infection. Similar findings have been made in small groups of pregnant (17) and postpartum women (19). In the present study 34% of 115 women with urinary tract infection showed more than 25% improvement of the concentrating ability. The assumption that these patients had acute pyelonephritis is supported by the presence in most of them of other signs of acute pyelonephritis. Among the patients with normal concentrating ability and no significant changes in MCA during the observation period one showed clinical symptoms of acute pyelonephritis while the coli antibody titre was increased in a few cases.

Patients with persistent and unchanged reduction of MCA are assumed to have chronic pyelonephritis, while significantly progressive reduction of MCA during the observation period may be due to intercurrent acute pyelonephritis or to established chronic pyelonephritis.

The recurrence rate was nearly the same in patients with initial MCA above 800 mOsm/kg and in patients with initial MCA below 800 mOsm/kg but significantly higher among patients with persistently lowered MCA or progressive reduction of MCA than in patients with normal or improving MCA. It is concluded that changes in MCA are associated with recurrent infection but the findings do not indicate that chronic pyelonephritis predisposes to reinfection of the urinary tract. Is seems more likely that a high number of recurrences entails a high frequency of chronic pyelonephritis.

## REFERENCES


1. Addis, T. & Foster, M. G.: The specific gravity of the urine. Arch. intern. Med. 30: 555, 19...
2. Baldwin, D. S., Berman, H. J., Heinemann, H. O. & Smith, H. W.: The elaboration of osmotically concentrated urine in renal disease. J. clin. Invest. 34: 800, 1955.
3. Brod, J., Prát, V. & Dejar, R.: Early functional diagnosis of chronic pyelonephritis with some remarks on the pathogenesis of the pyelonephritic contracted kidney. In: Biology of pyelonephritis, p. 311. E. L. Quinn and E. H. Kass, eds. Little, Brown & Co, Boston 1960.
4. DeWardener, H. E.: Vasopressin tannate in oil and the urine concentration test. Lancet 1: 1037, 19 6.
5. Elder, H. A. & Kass, E. H.: Renal function in bacteriuria of pregnancy. Its relationship to prematurity, acute pyelonephritis, and excessive weight gain. In: Progress in pyelonephritis, p. 81. E. H. Kass, ed. F. A. Davis Co., Philadelphia 1965.


Table II. *Number of patients with recrudescence or reinfection during 12 months' observation shown in relation to changes in MCA*

| Changes in MCA in relation to initial value | Persistent bacteriuria | Recrudescence | Reinfection | Sterile urine for 12 mo | Total |
|---|---|---|---|---|---|
| Improved by more than 25% | 0 (0 ) | 2 (5 ) | 9 ( 4 ) | 6 ( 0 ) | 37 |
| Initial MCA above 800, changed by less than 25% | 1 (3 ) | 4 (12* ) | 7 (21 ) | 22 (65 ) | 34 |
| Initial MCA below 800, changed by less than 25% | 1 (3 ) | 6 (18 ) | 13 (39 ) | 13 (39 ) | 33 |
| Deteriorated by more than 25 | 0 (0 ) | 2 (25 ) | 3 (38* ) | 3 (38 ) | 8 |

6 Epstein, F H Carone F A., Levitin, H., Manitius, A & Beck, D Loss of concentrating power and its relationship to pathology of the medulla in some experimental and clinical diseases of the kidney In Biology of pyelonephritis, p 293 E. L. Quinn and E. H. Kass, eds. Little Brown & Co Boston 1960

7 Fairley K. F Bond, A. G., Brown R B & Habersberger P Simple test to determine the site of urinary tract infection. Lancet 2 427 1967

8 Frank, M N Dreifus, L. S Ranck, F & Bellet S. Urinary osmolar concentration in the hydropenic state as a measure of renal tubular function a test for early renal impairment. Amer J med. Sci 233 1 1957

9 Kaitz, A. L. Urinary concentrating ability in pregnant women with asymptomatic bacteriuria. J clin. Invest 40 1331 1961

10 Kaitz, A. L. & London, A. M Osmolar urinary concentrating ability and pyelonephritis in hospitalized patients. Amer J med. Sci. 248 7 1964

11 Kleeman, C R., Hewitt, W L. & Guze L. B Pyelonephritis. Medicine (Baltimore) 39 3 1960

12 Lewis, W H. & Alving, A. S.. Changes with age in the renal function in adult men. Amer J Physiol. 123 500 1938

13 Mabeck, C E Studies in urinary tract infections. I The diagnosis of bacteriuria in women. Acta med scand. 186 35 1969

14 — Studies in urinary tract infections. II. Urinary tract infections due to coagulase negative staphylococci Acta med scand. 186 39 1969

15 Miles, B E., Paton, A & DeWardener H E Maximum urine concentration. Brit. med J 2 901 1954

16 Neter E., Bertram, L. F., Zak, D A Murdock, M R. & Arbesman, C E Studies on hemagglutination and hemolysis by Escherichia coli antisera J exp Med. 96 1 1952.

17 Norden, C W & Tuttle E P Impairment of urinary concentrating ability in pregnant women with asymptomatic bacteriuria In Progress in pyelonephritis, p 73 E. H Kass, ed F A Davis Co Philadelphia 1965

18 Raaschou, F Dissociation between glomerular and tubular renal function in cases of pyelonephritis. Acta med. scand 114 414 1943

19 Seligman, S J & Hewitt W L Urinary concentration test on postpartum bacteriuric women In Progress in pyelonephritis, p 558 E H Kass, ed F A Davis Co Philadelphia 1965

20 Stamey T A Govan, D E & Palmer J M The localization and treatment of urinary tract infections Medicine (Baltimore) 44 1 1965

21 Winberg, J Determination of renal concentration capacity in infants and children without renal disease Acta paediat (Uppsala) 48 318 1959

22 — Renal function studies in infants and children with acute nonobstructive urinary tract infections. Acta paediat (Uppsala) 48 577 1959

# Congress Announcement

*International Courses in Health Development* will be held in Amsterdam the Netherlands from September 1970 onwards and in Antwerp Belgium from September 1971 onwards (both courses ten months)

*Participation* is open to graduates in medicine with at least four years experience in developing countries and with proved managerial responsibilities in medical and health services

*Information* from the Netherlands Universities Foundation for International Co-operation (NUFFIC) 27 Molenstraat The Hague the Netherlands or from the Belgian Office for Development Co-operation (OCD) 5 Marsveldplein Brussels 5 Belgium

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# FISTULA OF THE THORACIC DUCT AS IMMUNOSUPPRESSIVE TREATMENT IN RHEUMATOID ARTHRITIS

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*Abstract* Six patients with severe, progressive classical rheumatoid arthritis and one with progressive polyarthritis of an active juvenile type have been treated by drainage of the thoracic duct. The lymph was kept flowing for seven days in four patients. In one case the drainage tube was occluded after five days, and in another the lymph flow had to be stopped after three days because of a psychical reaction. The total volume of lymph drainage ranged from 2.1–14.5 l, the cell mass from 400–1 400 × 10⁸ The immediate response to treatment was good in three patients, fairly good in two while no effect was obtained in the patient showing the juvenile type of the disease In four out of six patients the remission lasted for several months as shown by follow up examination. Cytostatic treatment was administered when these patients showed obvious exacerbation. All four responded well to the treatment.

It has been convincingly shown that the immune system plays an important part in the pathogenesis of systemic connective tissue disorders The beneficial effect of glucocorticoids as an immunosuppressive treatment in some patients is well known Cytostatics have been used with good results in rheumatoid arthritis (2 7) Antilymphocyte serum has been tried in systemic lupus erythematosus (SLE) (10)

Two patients with SLE responded well to drainage of the thoracic duct (11) Lymph drainage has been tried as an immunosuppressive measure in connection with grafting of skin in rats (3) and kidney transplantation in dogs (14) and man (4) The reduction of the lymphocyte pool and the circulating proteins by means of a thoracic duct fistula has not been hazardous to the patients (1)

There seemed to be reason to make a trial with lymph drainage as an immunosuppressive treatment in severe progressive rheumatoid disease before starting treatment with cytostatics

## MATERIAL AND METHODS

During the period 1966–1969 six patients with severe progressive rheumatoid arthritis from the Rheumatism Foundation Hospital Heinola were chosen among a number who volunteered for drainage of the thoracic duct The classification of the disease is seen in Table I Volunteers with severe renal and or hepatic impairment were rejected The patients were told not to expect any dramatic results of the drainage and no beneficial effects were promised

The operation was performed as previously described (1) The fistula was kept open for seven days if possible No complications occurred except in one case (no 1) in which the tube occluded spontaneously after five days of lymph flow In another patient (no 5) the tube was ligated and removed after three days because the patient showed increasing haematocrit values and refused to take intravenous infusions She developed a psychic reaction which lasted for only a couple of days

After the first day of drainage the patients were allowed to eat and move about The doses of antiphlogistics and/or glucocorticoids were not increased as compared to those given before operation

The lymph was collected in sterile closed bags and refrigerated immediately after removal

Every morning throughout the drainage the total and differential leucocyte counts and the number of lymphocytes in the lymph were recorded In three cases the lymph lymphocytes were differentiated by size The electrolyte total serum protein and haematocrit values were continuously recorded in blood and lymph The gammaglobulins were estimated by paper electrophoresis and the immunoelectrophoretic protein separation in serum and lymph was checked Rheumatoid factor (RF) was titrated by the Waaler-Rose and latex fixation tests in serum and lymph In addition LE-cell tests were performed and nucleic acid antibodies, antistreptolysin and uric acid were determined

In the serum and lymph of patient no 4 separations of IgG IgA, IgM and $\beta$ 1-C/$\beta$ 1 A (complement factor 3) was performed by the radial immunodiffusion technique (8) RF was titrated in the lymph cell suspension before and after homogenization Immunoelectrophoretic determinations for IgG IgM and IgA were made in the cell homogenate

Table I *The patient material*

| Pat no | Sex | Age | Description of disease | Duration of drainage | Total lymph flow |
|---|---|---|---|---|---|
| 1 | ♀ | 30 | Very active seronegative progressive malignant destructive classical rheumatoid arthritis | 5 | 4 492 (vacuolated lymphocytes) |
| 2 | ♂ | 31 | Very active seropositive erosive progressive classical rheumatoid arthritis with occasional positive LE cell phenomenon antinucleic acid antibodies prediabetes and impaired renal function (biopsy no lupoid changes) other systemic signs lacking | 7 | 14 460 |
| 3 | ♀ | 45 | Very active seropositive erosive progressive classical rheumatoid arthritis with occasional positive LE cell phenomenon and microscopic haematuria (biopsy no lupoid changes) other systemic signs lacking | 7 | 6 400 |
| 4 | ♀ | 40 | Very active seropositive malignant progressive destructive classical rheumatoid arthritis | 7 | 6 800 |
| 5 | ♀ | 23 | Very active seropositive progressive destructive classical rheumatoid arthritis | 3 | 2 100 |
| 6 | ♂ | | Juvenile type of progressive polyarthritis (onset at the age of 15) with peripheral erosive changes cervical and sacro iliac involvement seronegative | 7 | 9 370 |

During the first day of drainage specimens of blood and lymph were collected for testing the activities of creatinephosphokinase (CPK) glutamic-oxalacetic transaminase (GOT) glutamic pyruvic transaminase (GPT) lactic dehydrogenase (HBD) alkaline phosphatase acid phosphatase and aldolase

## RESULTS

. second and third day of drainage a striking improvement of the symptoms occurred in five out of six patients Morning stiffness of the joints pain and tenderness disappeared almost completely in three cases The immediate reaction to the drainage was remarkable in two patients The patient who showed the juvenile type of rheumatoid dis ase did not respond (Table II) In all favourable cases the improvement continued as long as the lymph was flowing A follow up examination revealed a persistent remission in four patients (Table II) The b st results were obtained in patient no 2 who showed remission for over a year and did not need any kind of treatment Administration of cytostatics to these patients which was started when the first symptoms of relapse occurred had a beneficial effect

Two patients (nos 2 and 3) occasionally showed a positive LE-cell phenomenon (Table I) Both of them responded well initially (Table II) but only one had a persistent remission

The Waaler-Rose titres and the latex fixation tests were identical in lymph and serum A decrease of the erythrocyte sedimentation rate was noted in all patients during the first postoperative week A decrease in total serum proteins (Fig 1) and gammaglobulins (mean 35 %) was noted The lymph globulins showed the same immunoelectrophoretic pattern as the globulins in serum approximated to a concentration of one fifth of the serum concentration In one patient the IgG IgA and IgM values in serum decreased from 2 240 600 and 260 mg/100 ml before drainage to 1 360 420 and 192 mg/100 ml after drainage On the first day of drainage the values in lymph were 132 250 and 55 mg/100 ml Complement factor 3 did not decrease the values were 84 before cannulation and 94 mg/100 ml after The value in lymph was 55 mg/100 ml No immunoglobulins were detected by immunoelectrophoretic separation in homogenates of washed thoracic duct lymphocytes

The lymph cells about 80 % of which were small reached total values of 400–1 400 × 10$^9$

Table II *Results of treatment*

| Pat. no | Immediate response to treatment | Follow up examination |
|---|---|---|
| 1 | Good<br>Morning stiffness pain and tenderness disappeared during the first two days | During three years of careful follow up no signs of progression have been observed Disease activity decreased stead ly during cytostatic medication |
| 2 | Good<br>Morning stiffness pain and tenderness disappeared on the second day | Remission of locomotor symptoms lasted over one year No drugs were needed during this period Subjective symptoms (pain and morning stiffness) reappeared one year and seven months after drainage cytostatic med cation was started |
| 3 | Good<br>Morning stiffness pain and tenderness disappeared almost completely during the first three days | Remission of symptoms for eight days after which progression of the disease continued despite treatment |
| 4 | Fairly good<br>Morning stiffness disappeared during the first few days Pain and tenderness were sl ghter than before operation | Symptoms reappeared a couple of weeks after drainage Cytostatic medication with dramatic effect was started No progression of the disease during the follow up period of one year and eight months |
| 5 | Fairly good<br>Morning st ffness pain and tenderness disappeared almost completely but returned the day after closing the drainage tube Cytostatics were administered | Remiss on of symptoms lasted for s x months during which cytostatic medication was given Successful tapering of steroids |
| 6 | None<br>The treatment had no effect on the symptoms | No effect of drainage or the subsequent cytostatic treatment was noted during two years of follow up |

cells on the day before the flow was stopped (Fig 2) The blood lymphocytes maintained their initial levels (Fig 3)

The aldolase values obtained in lymph were throughout higher than the corresponding blood values (Fig 4) Extremely high aldolase activity was noted in the patient with the juvenile type of polyarthritis No significant differences were detected between the remaind r of investigated enzyme activities in serum and lymph

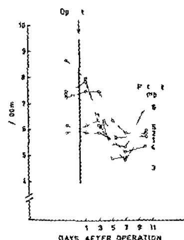



*Fig 1* The total serum proteins (g/100 ml) during drainage by thoracic duct fistula.

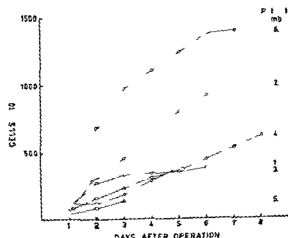



*Fig 2* Cumulative count of drained thoracic duct lymphocytes

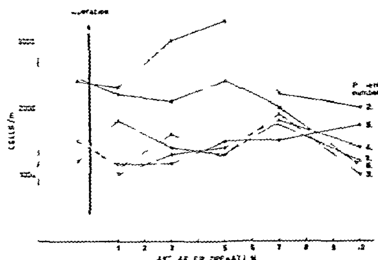



*Fig. 3.* Concentration of peripheral blood lymphocytes during drainage by thoracic duct fistula.

## DISCUSSION

The good response to thoracic duct drainage is not readily evaluated. The immediate disappearance of symptoms can be attributed to unspecific changes in the electrolyte protein and fluid balance as well as to psychological factors. The improvement of joint swelling and movement and the persistent remission in some cases are suggestive of a specific immunological effect of the treatment on the course of the disease. The important role played by the immune system in systemic connective tissue disorders has been convincingly demonstrated, but the mechanisms at a ar and molecular level remain obscure. The progress in immunology has increased the ...ing of many pathogenic events. Especially the classification of immuno-competent cells into thymus-dependent (T lymphocytes) and thymus independent (B lymphocytes) cells offers a good theoretical ground for further clinical investigations (12). The production of specific antibodies has been attributed to plasma cells deriving from B lymphocytes. The less well known T lymphocytes with immunological memory and a long life span exhibit cytotoxic abilities against graft target cells. In addition, they influence vascular permeability, activate macrophages and play a part in cell-mediated hypersensitivity. These small long-lived lymphocytes constitute the major part of the cell population in thoracic duct lymph. A diminution of this cell mass by thoracic duct cannulation has been effective as an immunosuppressive treatment in grafting and transplantation (3, 4, 14). A remission of severe rheumatoid disease by drainage of lymph as demonstrated in this study may be due to a decrease of the T lym-

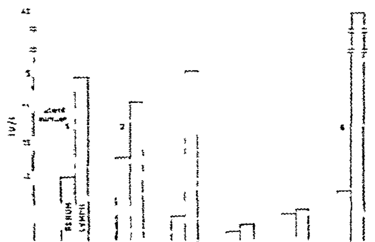



*Fig. 4.* The activities of aldolase in serum and lymph on the first day of drainage by thoracic duct fistula.

phocyte pool A decrease of peripheral blood lymphocytes was obvious even on the second postoperative day in 14 cases of renal transplantation (16) In our patients with systemic connective tissue disorder no blood lymphocytopenia could be observed during the first week in spite of the enormous cell mass removed (Fig 2) This may suggest a greater lymphocyte pool in patients with rheumatoid arthritis.

The small joint and buffy coat lymphocytes from patients with rheumatoid arthritis have been shown to exhibit cellular toxicity in fibroblast cultures (6 15) This in vitro observation may have some relevance in vivo too and explain the beneficial effect of the cannulation

In addition it seems possible that the decrease of B lymphocytes by a thoracic duct fistula influences the course of rheumatoid disease After conversion into plasma cells the B lymphocytes produce antibodies at the sites of inflammation especially in the synovial membranes (9) The circulating antibodies in part deriving from these sources decrease during lymph drainage and may influence the disease activity However no convincing evidence concerning tissue or cell toxicity of the rheumatoid factor has so far been reported Removal of cells alone and reinfusion of cell free lymph has proved effective as immunosuppression (13) In addition patients with SLE do not show any remission of immunoglobulins lost during a nephrotic episode (11) All these data indicate that the effect is caused by the reduction of immunocompetent cells rather than of circulating antibodies

The good results of cytostatic treatment in transplantation on the one hand and in rheumatoid arthritis on the other suggest the possibility that the T lymphocytes are the target of the effect A similar response has been obtained with anti lymphocyte serum in SLE (10) and in kidney transplantation

The results of this clinical study bear out the importance of immunological events the cell mediated reactions in the pathogenesis of rheumatoid arthritis in particular

No further conclusions concerning the rheumatoid pathogenesis can be drawn from enzymatic immunological or serological comparisons between the serum and lymph The aldolase findings remain without logical explanation That the concentration of aldolase in general was much higher in lymph than in serum may be a physiological phenomenon (1) or it may be accounted for by the importance of lymph drainage of the articular system In the joint fluid from patients with rheumatoid arthritis elevated aldolase levels have been noted indicating an activated glycolytic metabolism (5)

The beneficial effect of this kind of treatment and the absence of complications allow a further trial to be made in selected cases of connective tissu disorders. Extracorporeal irradiation of circulating thoracic duct lymphocytes may be a future immunosuppressive method of treatment

## REFERENCES


1 Blomstrand, R Franksson, C & Werner B The transport of lymph in man. Appelbergs Boktryckeri AB Uppsala 1965
2 Fosdick, W M Parsons, J L & Hill O F Long term cyclophosphamide therapy in rheumatoid arthritis. Arthr and Rheum. 11 151 1968
3 Franksson C Survival of homografts of skin in rats depleted of lymphocytes by chronic drainage from the thoracic duct. Lancet 1 1331 1964
4 — Transplantationskirurgi hos manniska Svenska Lak. Tidn. 6_ 93 1965
5 Greiling, H Selected problems in rheumatology International Symposium on Rheumatology Zermatt 1969
6 Hedberg, H Studies on synovia flu d in arthritis II The occurrence of mononuclear cells with in vitro cytotoxic effect. Acta med scand Suppl 4 9 1967
7 Lorenzen I. Brun C & Videbaek Aa Treatment of immunologic diseases with cytostatics Acta med. scand. 185 501 1969
8 Mancini, G Carbonara A O & Heremans, J F Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry _ _35 1965
9 Mellors R C Heimer R. Corcos, J & Korngold L. Cellular origin of rheumatoid factor J exp Med. 110 875 1959
10 Nossa, L. Muresan, T Mar_u, A. & Abrudeanu O Preparation of antihuman lymphocyte serum by immunization with un table lymphoid cells obtained from a cadaver and stored for 3 months in a frozen state Nature (Lond) ___ 1082, 1969
11 Olhagen, B & Franksson C Drainage from the thoracic duct as an immunosuppressive method. International Congress of Rheumatology Mar del Plata 1965
12 Roitt, I. M Greaves, M F Torrigiani G Brostoff, J & Playfair J H. L. The cellular basis of immunological responses. A synthesis of some current views. Lancet _ 367 1969
13 Sarles, H E., Smith G H Fish, J C & Re___ A R Jr Observations concerning human


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homeostasis during prolonged thoracic duct lymph diversion Tex Rep Biol Med 25 573 1967
14 Singh L. M Bega R. E Makin G S & Howard J M External thoracic duct fistula and canine renal homograft. J Amer med Ass 191 1009 1965
15 Stanfield A B Stephens C A L. Jr & Hill D F Tissue culture investigations in rheumatoid arthritis In Cellular concepts in rheumatoid arthritis C A L. Stephens, Jr and A B Stanfield eds Thomas Springfield 1966
16 Tilney N L. & Murray J E. Chronic thoracic duct fistula operative technic and physiologic effect in man Ann Surg 167 1 1968

SUBJECT INDEX

*(Supplements, see page XII)*

diseases



Diabetes mellitus

rtension



**Infection**



**Intoxication**

**Kidney**



**Liver**



**Lung**



**Metabolism**